Cerebrospinal fluid biomarkers and cognition in older adults

Maya Arvidsson Rådestig

Department of Department of Psychiatry and Neurochemistry,
Institute of Neuroscience and Physiology
Sahlgrenska Academy, University of Gothenburg

UNIVERSITY OF GOTHENBURG

Gothenburg 2021

Cerebrospinal fluid biomarkers and cognition in older adults

Maya.arvidsson.radestig@neuro.gu.se

ISBN 978-91-8009-320-0 (PRINT)
ISBN 978-91-8009-321-7 (PDF)

Printed in Gothenburg, Sweden 2021
Printed by Stema Specialtryck AB, Borås

ABSTRACT

Alzheimer's disease (AD) is the most common type of dementia. The cause of the disease is unknown, but a substantial amount of evidence suggest that the disease is started by an imbalance in the production and clearance of the protein amyloid-β, which then starts a cascade of neurodegenerative events leading to cognitive symptoms. Biomarkers in cerebrospinal fluid (CSF, amyloid-β and tau) reflecting the pathological changes in AD can be seen as early as 10-20 years before the clinical diagnosis. Pathological levels of the known CSF AD biomarkers in cognitively normal individuals can be seen frequently in older adults and this is called preclinical AD. This thesis explores different CSF biomarkers for AD and cognition in older adults in the preclinical phase of AD using a research framework classification system for preclinical AD – the so called ATN system.

Study I explores cognition in older adults with preclinical AD, using the ATN classification system. We investigated the hypothesis that individuals with positive AD biomarkers would have lower cognitive test scores than those without biomarkers, and we show this for some tests primarily in the memory domain. However, the groups with and without biomarkers performed similarly in most tests.

Study II investigates the relationship between cognition and the neurodegeneration biomarker neurofilament light protein (NfL) and the synaptic degeneration biomarker neurogranin (Ng). We investigated the hypothesis that individuals with high levels of the biomarkers NfL and Ng would have subtle cognitive decline compared to those with normal levels of these biomarkers. We found that this was true in a few cognitive tests, however, in most of the tests the groups with and without high levels of NfL and Ng performed similarly.

Study III investigates the levels of the biomarkers NfL and Ng in the ATN groups in cognitively normal older adults. We found that individuals with neurodegeneration (tau pathology) had higher levels of NfL and Ng, but we did not find any difference in NfL or Ng levels in participants with amyloid pathology compared to those without amyloid pathology. This suggests that older adults with T-tau pathology have increased axonal and synaptic damage.

In conclusion, the results from this thesis suggest that the known CSF biomarkers for AD and synaptic and neurodegeneration could be valuable biomarkers in the earliest phase of AD.

Keywords: preclinical Alzheimer's disease, cerebrospinal fluid, biomarkers, cognition

ISBN 978-91-8009-320-0 (PRINT)
ISBN 978-91-8009-321-7 (PDF)

SAMMANFATTNING PÅ SVENSKA

Alzheimers sjukdom är den vanligaste typen av demens. Det är oklart vad som orsakar sjukdomen, men mycket bevis tyder på att den orsakas av en obalans i produktionen och nedbrytningen av proteinet β-amyloid, vilket sedan resulterar i en kaskad av neurodegenerativa händelser som leder till de kognitiva symptomen. Biomarkörer i cerebrospinalvätskan (β-amyloid och tau) reflekterar patologiska förändringar vid Alzheimers sjukdom som kan upptäckas så tidigt som 10-20 år innan diagnosen ställs. Patologiska nivåer av de kända biomarkörerna för Alzheimers sjukdom i cerebrospinalvätskan är vanligt hos äldre kognitivt normala individer och kallas för preklinisk Alzheimers sjukdom. Denna avhandling utforskar olika Alzheimer-biomarkörer och kognition hos äldre i den prekliniska fasen med hjälp av ett klassifikationssystem för preklinisk Alzheimers sjukdom – det så kallade ATN systemet.

Den första studien utforskar kognition hos äldre med preklinisk Alzheimers sjukdom uppdelade i ATN-grupperna. Vår hypotes var att individer med biomarkörer för Alzheimers sjukdom har något lägre poäng på kognitiva tester i jämförelse med individer utan biomarkörer, och vi kunde visa detta för vissa kognitiva test, framförallt minnestest. Grupperna med och utan biomarkörer hade dock liknande poäng på de flesta kognitiva tester.

Den andra studien utforskar relationen mellan kognition och en biomarkör för neurodegeneration, neurofilament light (NfL) samt en biomarkör för synaptisk degeneration, neurogranin (Ng). Vi testade hypotesen att individer med högre nivåer av biomarkörerna NfL och Ng har något lägre poäng på kognitiva tester i jämförelse med individer med låga nivåer av biomarkörerna. Vi kunde visa att detta stämde för vissa kognitiva test, men grupperna med höga och låga nivåer av biomarkörer hade liknande poäng på de flesta kognitiva tester.

I den tredje studien jämförde vi nivåerna av biomarkörerna NfL och Ng i ATN grupper hos kognitivt normala äldre. Vi fann att individer med neurodegeneration (tau-patologi) hade högre nivåer av biomarkörerna NfL och Ng, men vi kunde inte påvisa någon skillnad i NfL eller Ng-nivåer hos delatagare med amyloid-patologi jämfört med de utan amyloid-patologi. Detta tyder på att äldre med tau-patologi har ökad förekomst av axonal och synaptisk skada.

Sammanfattningsvis, så tyder resultaten i den här avhandlingen på att de kända biomarkörerna för Alzheimers sjukdom samt biomarkörerna för synapsskada och neurodegeneration i cerebrospinalvätska, kan vara viktiga biomarkörer i den tidigaste fasen av preklinisk Alzheimers sjukdom.

LIST OF PAPERS

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

I. **Cognitive performance and cerebrospinal fluid markers in preclinical Alzheimer's disease: results from the Gothenburg H70 studies.**
Rådestig, A.,M., Skoog, J., Zetterberg, H., Kern, J., Zettergren A., Sacuiu, S., Waern, M., Wetterberg, H., Blennow, K., Skoog, I., Kern, S.
Journal of Alzheimer's Disease 79 (2021) 225–235

II. **Cognition in older adults with elevated CSF neurofilament light and neurogranin – a H70 cross-sectional study.**
Rådestig, A., M., Skoog, J., Zetterberg, H., Skillbäck, T., Zettergren A., Sterner, R.,T., Fässberg., M., M., Sacuiu, S., Waern, M., Wetterberg, H., Blennow, K., Skoog, I., Kern, S.
Manuscript

III. **Cerebrospinal fluid biomarkers of axonal and synaptic degeneration in a population-based sample.**
Rådestig, A.,M., Skoog, I., Skillbäck, T., Höglund., K., Zetterberg, H., Kern, J., Zettergren A., Andreasson, U., Wetterberg, H.., Blennow, K., Kern, S.
Manuscript

Paper I is reprinted with permission from the publisher.

CONTENT

1 INTRODUCTION 6
1.1 The Gothenburg H70 Birth Cohort Studies 6
1.2. Study sample - The 1944 cohort 6
1.2 Alzheimer's disease 7
1.3 Mild cognitive impairment 7
1.4 Neuropathology 8
1.4.1 Amyloid-β (Aβ) 8
1.4.2 Tau 9
1.5 The amyloid cascade hypothesis 10
1.6 Diagnosis 12
1.7 Risk- and preventive factors 14
1.8 Genetics 15
1.9 Preclinical Alzheimer´s disease 16
1.9.1 The ATN system 17
1.9.2 CSF Biomarkers 18
1.9.3 Amyloid-β_{42} ($A\beta_{42}$) 19
1.9.4 Phospho-tau (P-tau) 19
1.9.5 Total tau (T-Tau) 20
1.9.6 Neurofilament light (NfL) 20
1.9.7 Neurogranin (Ng) 21
2 AIM 22
3 METHODS AND STUDY DESIGN 23
3.1 Study sample – the Gothenburg H70 Birth Cohort Studies 23
3.1.1 The 1944 cohort 25
3.2 General examination and neuropsychiatric assessments 26
3.2.1 Cognitive examination 27
3.2.2 Memory tests 27
3.2.3 Language tests 28

3.2.4 Executive function and mental speed tests 28
3.2.5 Mini Mental State Examination and Clinical Dementia Rating..... 29
3.2.6 Cerebrospinal fluid sampling, genotyping and biomarker analyses 29
3.3 Ethical considerations 31
3.4 Statistical Analyses 31
4 RESULTS 33
4.1 Study I 33
4.2 Study II 34
4.3 Study III 35
5 DISCUSSION 36
5.1 Interpretation of the main findings 36
5.2 Subtle cognitive decline can be seen in groups with neurodegeneration 36
5.3 How does neurodegeneration relate to cognition? 38
5.4 Possible causes of synapse pathology in AD 39
5.4.1 Plaques and amyloid-β 39
5.4.2 Tau and neurofibrillary tangles 41
5.4.3 Amyloid and tau oligomers and synaptic dysfunction 42
5.5 Episodic memory and language in early AD……...43
5.6 Summary 44
5.7 Methodological considerations 44
5.8 Statistical considerations 46
5.9 Strengths and limitations 46
5.10 Clinical implications 47
5.10.1 The utility of early AD diagnosis 47
5.11 Controversies 48
5.11.1 Presymptomatic testing 48
6 CONCLUSION 49
7 FUTURE PERSPECTIVES 50
ACKNOWLEDGEMENT 51
REFERENCES 53

ABBREVIATIONS

AD – Alzheimer's disease

ADAD - autosomal dominant Alzheimer's disease

APOE Apolipoprotein E

APP – Amyloid Precursor Protein

CDR – Clinical Dementia Rating

CSF – Cerebrospinal fluid

DSM – Diagnostic and Statistical Manual of Mental Disorders

ELISA - enzyme-linked immunosorbent assay

FDG-PET – Fluorodeoxyglucose PET

MCI – Mild Cognitive impairment

MMSE – Mini Mental State Examination

PET – Positron emission tomography

PSEN – presenilin

DEFINITIONS IN SHORT

Dementia	A general term describing conditions with symptoms relating to impaired memory and cognitive functions, that interfere with daily living.
Alzheimer's disease	An incurable neurodegenerative disease characterized by impairment in memory and other cognitive functions.
Preclinical Alzheimer's disease	A term used in research settings describing the stage when biomarkers of Alzheimer's disease are present in cognitively normal individuals.

INTRODUCTION

1.1 THE GOTHENBURG H70 BIRTH COHORT STUDIES

The Gothenburg H70 Birth Cohort Studies began in 1971 and include several representative birth cohorts of older adults that have been followed for decades. It is a population based multi-disciplinary study that comprises numerous comprehensive examinations on somatic, psychological and cognitive health, as well as lifestyle and social factors. The examinations have been identical over time, which provides a valuable opportunity to examine how aging and health changes have developed through the decades, as well as identifying biomarkers and risk-/protective factors for aging related diseases. The overall aim of the H70 studies is to study the effects of psychological and physical health on well-being in old age and to improve the capability of older adults, which can be defined as an individual's ability to fulfil goals that he or she considers valuable. Studies on aging can help inform policies and recommendations which may lead to improved health care and services for older adults, as well as increased well-being, which is dependent on mental and physical health. A better understanding of aging and aging-related diseases is important as the number of older adults is increasing [1]. The H70 study is one of few population based studies in the world, and using a population-based sample can minimize the potential bias that may result from convenience samples and recruitment of volunteers.

1.2. STUDY SAMPLE - THE 1944 COHORT

The cohort used in this project was born in 1944 and examined during 2014 to 2016[2]. All participants were approximately 70 years old at the time of examination. More detail about the sample are given in the methods section.

1.2 ALZHEIMER'S DISEASE

Alzheimer`s disease (AD) is a progressive incurable dementia characterized by gradual loss of cognitive abilities and independence [3]. It is the most common cause of dementia, and old age is the most prominent risk factor [3]. In 2015 there were over 46 million people living with dementia globally and this number almost doubles every 20 years[4]. AD accounts for 50-75% of the dementia cases [5]. The disease progression varies between individuals, and the earliest phase can be difficult to distinguish from normal aging related memory decline. In normal aging, procedural and semantic memory is often well preserved, whereas episodic and working memory and executive functions can decline [6]. In AD, memory impairment, especially in episodic memory, is one of the first symptoms [7]. Other cognitive functions that can be affected early include visuospatial ability, language, executive function, semantic memory and global cognition [7-13]. Depressed mood and behavior symptoms can also be seen [9]. As the disease progresses, individuals with AD experience a gradual cognitive decline and a difficulty to function independently in daily life. Symptoms such as aphasia, apraxia, agnosia, and impairment in judgement, decision-making and orientation are common [3]. Patients can also show behavioral symptoms such as aggression and psychosis [3]. Most people live with AD for around 8 years on average [14]. Despite numerous clinical trials in the last few decades, there is still no disease-modifying treatment [5]. The treatments available today can modestly improve the cognitive symptoms but not halt the disease progression [5,14].

1.3 MILD COGNITIVE IMPAIRMENT

Mild cognitive impairment (MCI) is often considered to be a state between normal aging and dementia [15-17]. The concept of MCI has existed for more than 2 decades and is still evolving [16]. MCI patients have mild symptoms, usually related to memory or to performing complex tasks, but they still function independently in daily life [16], and can have high MMSE scores [15]. The concept of MCI assumes a decline from a previous level of cognitive function [16]. Some patients have MCI due to causes unrelated to dementia [16,17] and it may be a stable condition but studies have shown that individuals with MCI progress at

a considerably higher rate than healthy controls to dementia [18]. In one study, among MCI patients, around half had converted to dementia after 5 years, and all of them converted after around 10 years [15]. MCI can be divided into different subtypes, depending on which cognitive domain is most affected [17,18]. In clinical trials the Clinical Dementia Rating score (CDR) is often used. This score is calculated based on six different domains (memory, orientation, judgment and problem solving, community affairs, home and hobbies performance, and personal care[19]). Individuals with a global CDR score of 0.5 frequently have MCI or mild AD [18], and CDR0.5 can therefore sometimes be used as a proxy for MCI.

1.4 NEUROPATHOLOGY

AD is characterized by the hallmark brain lesions plaques and tangles, deposited in the medial temporal lobes and in the cortex [3,20], and by loss of neurons and synapses [3]. Plaques are extracellular depositions consisting of aggregated proteins, mainly amyloid β (Aβ). Neurofibrillary tangles, consisting of hyper-phosphorylated tau, aggregate inside neurons where they cause damage and possibly cell death [3,21]. Plaques and tangles are the cardinal neuropathological changes in AD, but they can also be present in cognitively normal older adults [3,22,23]. Mixed pathology is also common in AD [24], and ”pure” AD is rare in older dementia patients [20].

1.4.1 AMYLOID-β (Aβ)

An amyloid is a self-assembled protein aggregate characterized by β-sheet structures [25]. Amyloids are present in many incurable neurodegenerative diseases [25] and their peptide composition varies with the disease [14]. In AD, amyloid plaques consist of several types of peptides, the most well-known and studied being Aβ[26]. Aβ depositions are present in the neocortex, the hippocampus and in the vasculature of the brain in AD [26]. Aβ peptides are by- products of normal brain cell metabolism (cleavage of the transmembrane protein amyloid precursor protein (*APP*) [26]). There are two pathways for cleavage of *APP*, a non-amyloidogenic pathway where *APP* cleavage does not result in Aβ deposition, and an amyloidogenic pathway that generates Aβ [3]. The initiating factor in AD pathology is considered to be an imbalance in

the production and clearance of Aβ [3,27], although the downstream details of this imbalance have been discussed, and exactly what role Aβ fibrils (plaques) play in AD etiology remains a subject of debate. Besides from forming large insoluble fibrils, Aβ also exists in an oligomeric form, which is smaller and believed to be neurotoxic, and may affect synapses [14]. Aβ exists in different isoforms, or fragments with a different number of amino acids. The 42 amino acids long variant is the most cytotoxic and aggregation prone, probably due to its hydrophobicity [28-30].

1.4.2 TAU

Tau's most well-known function in a healthy cell is to stabilize microtubules – the transport system of the cytoskeleton [31,32]. During the AD progression, tau becomes hyper-phosphorylated, detaches from the microtubules, leading to their disassembly, and accumulates in paired helical filaments [3,29,33] as well as loses its normal function [24,32]. This could lead to neurodegeneration.[32] (Tau may also be involved in other activities than microtubule-stabilization, since it has many binding partners, including signaling molecules [31].) Tau also undergoes several other post translational modifications, such as glycosylation, glycation, nitration, ubiquitination and truncation [34]. It is not clear how all of these modifications relate to tau pathology, but some of them might lead to increased tau aggregation [34] and production of oligomers [35]. Tau is normally localized in the axon, but can also be found in synapses [36]. Tau pathology in AD spreads in a characteristic pattern through the brain, starting in the transentorhinal region, and then spreads to the hippocampus and amygdala, which are involved in memory functions, and through the neocortex with increasing pathology in the later stages of the disease [37,38] (Figure 1). Tau pathology is more strongly correlated with cognitive decline than Aβ [39]. However, the role of tau in causing neurodegeneration remains unclear. [40]

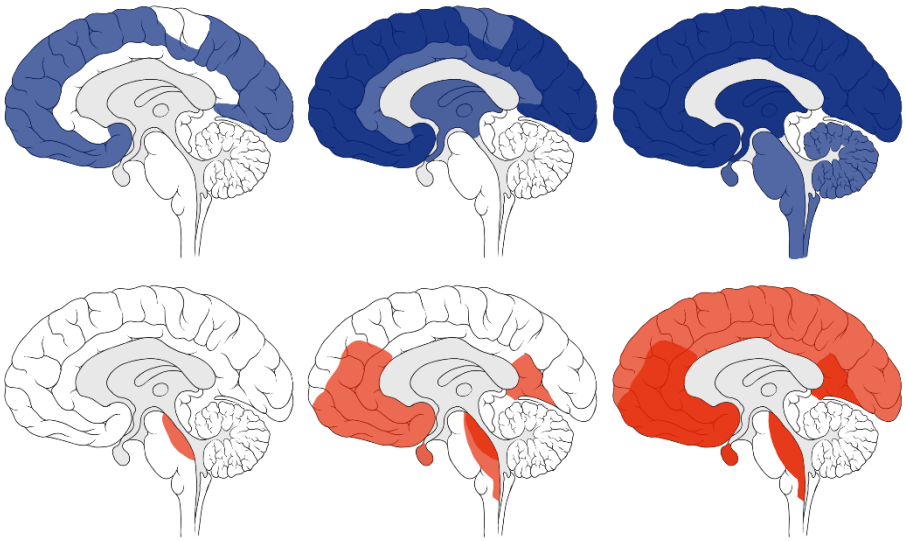

Figure1. The propagation of amyloid and tau pathology in Alzheimer's disease. Aβ plaques are shown in blue and neurofibrillary tangles are shown in red. Image courtesy of Tobias Skillbäck[41].

1.5 THE AMYLOID CASCADE HYPOTHESIS

The cause of AD is still unclear, despite the fact that the cardinal symptoms have been known for more than 100 years, and there are many hypotheses regarding the etiology [42,43]. The amyloid cascade hypothesis has been central to AD research for the past few decades, and states that the deposition of amyloid-β fibrils (plaques) in the brain parenchyma triggers a cascade of neurotoxic molecular events, including tau-pathology, in early AD [44,45]. Later follows neurodegeneration and subsequent loss of cognitive and mental functions [42] (Figure 2). There is now strong evidence regarding this sequence of events [27,46,47]. The original amyloid cascade hypothesis stated that amyloid is cytotoxic[42,48-50] and has a driving role in pathogenesis [45]. This, however, has been debated, and many

researchers now believe that downstream actors in the cascade, such as tau or oligomers, are more cytotoxic than fibrillar amyloid and may have a more important role in causing neurodegeneration. The amyloid cascade hypothesis has been updated in order to take this into consideration [44]. There has also been some skepticism regarding how neurotoxic fibrillary amyloid is [42,51], (which seems to depend on whether the plaques are neuritic or diffuse[22,48]). The interplay between amyloid and tau seems to be central to the cascade, but the details of their interaction is still not clear. Another explanation suggests that they could be linked by a common upstream driver that leads to separate pathways of Aβ and tau pathology [52].

There are also many other hypotheses about potential causes and mediators of AD [42,43], some of which are briefly reviewed below.

The tau propagation hypothesis has also been influential in AD research and in clinical trial design, and states that aggregates of misfolded hyper-phosphorylated tau spread from the outside to the inside of a cell [42,53]. Tau pathology starts in specific regions in AD and has been suggested to spread throughout the brain in a prion like fashion [54], which could affect signal transduction within and between neurons [42,53]. This propagation of tau may be responsible for causing neurodegeneration, although this has been debated [21,54].

Another hypothesis that has gained increasing attention suggests that oligomers of Aβ and tau, play a central role in AD pathogenesis and neurodegeneration. Oligomers (peptides consisting of a few amino acids) are neurotoxic [28,48,55]. They can inhibit long term potentiation which is important for memory formation[29,55,56] and cause learning deficits in animals [29,56]. Oligomers can also damage synapses [14]. They can be found early in the disease process [14].

Microglia, which are part of the brains immune system, may be implicated in AD pathogenesis but it is not clear how or to what extent [42,57]. Activation and proliferation of microglia is a characteristic of neurodegenerative diseases such as AD [57], but it is not clear whether they are beneficial or harmful, as studies have demonstrated both, and it depends on the context [43]. Microglia clear the brain of debris including Aβ, but they can also engulf synapses, damage neurons and cause neuroinflammation [57], which may contribute to disease pathogenesis [57].

Other alternative hypotheses about factors that may be involved in AD pathogenesis include disruption of lipid homeostasis [58], high

cholesterol levels [59] infectious pathogens [60], gut microbes[43], the complement system [61] mitochondrial dysfunction and oxidative stress [42,43]. However, for many of these hypotheses, the evidence have been inconsistent.

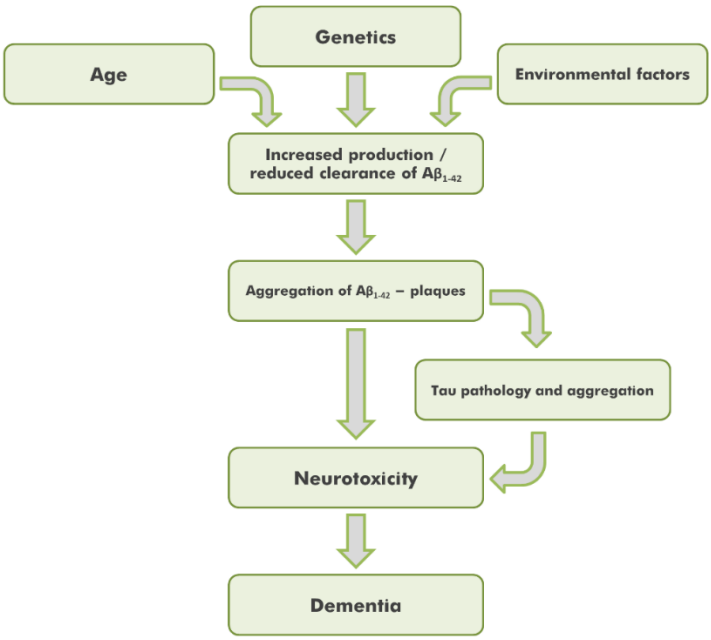

Figure2. Schematic of the amyloid cascade hypothesis. Image courtesy of Tobias Skillbäck[41].

1.6 DIAGNOSIS

The only way to definitely diagnose AD is by autopsy [20], since examination of the brains' microscopic features is required to determine the presence of AD-related neuropathologic changes (the macroscopic features such as atrophied cortex and enlarged sulci in certain areas are suggestive of but not specific to AD [24].) However, there is currently a debate about how to conceptualize the AD diagnosis, with many researchers arguing that it should encompass pathological biomarkers that appear long before the clinical

manifestation [62]. However, it is unclear how effectively biomarkers in cognitively normal people can predict progression [33]. The National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) have developed criteria for the diagnosis of AD used by researchers and clinicians that have proven successful and valid over the past few decades, since 1984 [63]. In 2011, these criteria have been revised by a workgroup from the National Institute on Aging – Alzheimer`s Association workgroups [63].

The NINCDS-ADRDA criteria for dementia are based on a patients decline from a previous level regarding their inability to function in daily life, (unless these symptoms can be explained by a psychiatric disorder) and cognitive decline, including at least two of the following areas: forgetfulness /impaired ability to acquire new information, poor judgement / decision making, impaired visuospatial ability, impaired language or personality change. The AD diagnosis include the above criteria for dementia, and also includes: gradual onset of symptoms, history of worsening of cognition, and cognitive deficits in one of the following areas: amnestic, non-amnestic (language, visuospatial ability, or executive function), and absence of evidence of differential diagnoses, such as cerebrovascular disease with a history of stroke, multiple infarcts with severe white matter hyperintensity, dementia with Lewy bodies or frontotemporal dementia [63].)

The clinical diagnosis of AD is based on a combination of clinical assessment where cognitive tests and informant interview are used [5]. CSF or brain imaging biomarkers (described below) and genetic tests can also be used as supportive criteria [22,33].

Diagnosis of AD is often based on the exclusion of other diseases [64]. There are many differential diagnoses, such as other dementias, which can be excluded using CSF biomarkers, or conditions such as brain tumor or subdural hematoma, which can be excluded by CT and MRI [3]. CSF biomarkers can also be used to exclude rare and reversible causes of cognitive decline [5].

In this project we have used the DSM III-R for dementia diagnosis, in order to make diagnoses comparable over time.

1.7 RISK AND PREVENTIVE FACTORS

Late onset AD is a complex disease that seems to result from a multifactorial interplay of genetic and environmental and life-style factors with increasing age being a very important risk factor [5,65,66]. Regarding genetic susceptibility, the *APOE* gene (discussed below) is the primary determinant [5]. Since many of the risk factors are life-style related, there is a potential for prevention, also if started later in life [1]. Some examples of modifiable factors with a protective effect include eating a Mediterranean diet [62,65,66], which is a diet rich in vegetables, fruit, fish and unsaturated fats and low in meat and dairy [66]. It is generally difficult to pinpoint the effect of specific foods or vitamins on AD risk, but a few examples that have been reported as protective include folate, vitamin E and C, and coffee [3,65,66], although the evidence of an association is not conclusive [3,66].

High education early in life and cognitive activities are also thought to decrease dementia risk [1,5,65,66]. This could be explained by the hypothesis of the cognitive reserve [1,67,68]. According to this hypothesis, there are two types of reserve; brain reserve, which means physical differences in the brains of individuals, and cognitive reserve, which refers to functional differences, and individuals with higher reserve are hypothesized to better tolerate pathology without manifesting symptoms [23,66-68]. Epidemiological studies have shown that educational and occupational attainment can increase the reserve [67]. Paradoxically, although high reserve results in delayed onset of symptoms, it is also associated with an acceleration of symptoms (faster cognitive decline) later in the disease progression [69]. But this apparent inconsistency can also be explained by the cognitive reserve hypothesis, since it suggests that of two individuals with the same level of clinical severity, and different levels of cognitive reserve, the person with higher reserve will have more pathology (although they are better able to cope with it). Thus, the higher level of cognitive reserve could only mask the clinical manifestation of AD, until the underlying pathology reaches a certain tipping point when the decline becomes more rapid[66].

Physical activity is also associated in many studies with lower dementia risk [1,66,70]. It has also been reported that having a large social network[1] and participation in leisure and cultural activities [66,71,72] is associated with lower dementia risk.

There are also potentially modifiable risk factors, many of which can

be managed or avoided in order to decrease the risk of AD. These risk factors include hypertension, excessive alcohol consumption, and smoking, which has been shown in some, but not all [65], studies to increase the AD risk [1,65,66]. Conditions such as diabetes, obesity, atherosclerosis, high cholesterol, cerebrovascular disease, head injury, hearing loss and hypertension in mid- and late life also increase the likelihood of developing AD, although it is not clear how, or whether they are directly related to AD (some are associated with vascular disease) [1,3,66]. Environmental exposures such as air pollution have also been reported as a risk factor[1]. Late life depression could also be associated with risk of AD, although the relationship could be bidirectional, since people with depression may engage less in protective activities such as cognitive and physical activities [1,65,66]. Theoretically, a lifestyle that includes the preventative factors and avoids the risk factors, may help lowering the risk of developing AD [1].

1.8 GENETICS

Although most cases of AD are sporadic, cases with autosomal dominant familial AD are known [3]. Familial AD is caused by a mutation in genes, amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*) and presenilin 2 (*PSEN2*) [73], both presenilin genes are linked to Aβ metabolism [49], and the disease is called autosomal dominant AD (ADAD). Patients with ADAD often present an early disease onset before the age of 65 years while patients with sporadic AD often have a later onset of disease [73].

For sporadic AD, *APOE ε4* is a very prominent risk factor [74]. It is a susceptibility gene, which exists in three variants, (ε2, ε3 and ε4) [74]. The mechanism whereby *APOE ε4* increases the risk for the development of AD is not fully known and probably involves many pathways. ApoE is involved in cholesterol homeostasis and thereby mediates neuronal protection and plays a role in the removal of Aβ plaques. ApoE *ε4* is the least efficient isoform, leading to increased accumulation in those who have one or two copies of this allele [74] *APOE ε4* decreases age of onset in sporadic AD by several years [75].

1.9 PRECLINICAL ALZHEIMER´S DISEASE

There is a phase spanning several decades before AD symptoms manifest where biomarker evidence of pathology are detectable on CSF and PET [23,27,76]. This led to the development of the concept of preclinical AD, in the late 20th century [62]. One of the most important questions in AD research is whether it is possible to diagnose or predict the risk of AD in this preclinical phase, opening up the possibility of early prevention strategies. This has been debated, with many researchers arguing that pathology on CSF before cognitive decline represents an ongoing disease process [77] or argues that preclinical AD could be a target for therapeutic intervention [78]. The preclinical phase is also important for studies on prevention of disease progression to a clinical phase [23,62]. The view that AD begins long before the clinical diagnosis is established has been supported by some studies that have shown a slight impairment in cognition already in the preclinical phase [12,79-81] and that AD biomarkers in this phase can predict progression [78]. Progressive cognitive impairment and pathological changes in biomarkers over a 25-year period before diagnosis have also been seen in individuals with autosomal dominant Alzheimer's disease [82]. However, the results have been mixed, and some studies have also failed to report an association between AD biomarkers and cognitive impairment in the preclinical phase [83-85]. There are also individuals with evidence of AD neuropathology at autopsy that never manifest cognitive symptoms during life [81,86]. Since it is not known whether these people would have developed AD eventually if they had lived longer [87], there is a controversy over whether it is most appropriate to label signs of amyloid and tau pathology in cognitively normal people risk factors for AD, or early evidence of the disease process itself [88,89].

However, the relationship between preclinical AD and cognition needs to be investigated further. There is a need for a deeper understanding of these issues since asymptomatic people may be included in clinical trials for disease-modifying drugs, and if such drugs are developed, treatment and screening of asymptomatic individuals could become a reality.

If it is possible to diagnose AD in the preclinical phase, another question would be how to develop suitable cognitive tests, and in which cognitive domains the slight impairment appears first or is most

evident.
Several criteria have been published for the research diagnosis of preclinical AD [62,76,81,90,91] and we have used Jacks ATN system which is the most recent and most common [76].

1.9.1 THE ATN SYSTEM

The ATN system is a research classification scheme for AD biomarkers developed by Jack et al [76]. It is frequently used in studies on AD and provides a common language for researchers in the AD field.
In the ATN system, the three core AD biomarkers are divided into binary categories: A T and N. A stands for amyloid pathology, T for P-tau pathology and N for neurodegeneration. High ligand retention on amyloid PET or low CSF $A\beta_{42}$ are biomarkers of brain amyloid deposition [76]. High CSF P-tau or high levels of tau on PET are biomarkers of neurofibrillary tangles [76]. Elevated CSF T-tau or hypo-metabolism on FDG-PET or atrophy on structural MRI in AD specific regions are biomarkers of neurodegeneration [76].
When applied to clinically normal individuals, the ATN system and the NIA-AA classification divides people into three stages; stage 1 is when an individual only has amyloid pathology, stage 2 is when a person has amyloid plus tau pathology, and stage 3 is when amyloid and tau pathology appear in combination with a slight cognitive decline [76,77]. Individuals in stage 2 and 3 are considered by the authors of the ATN system to have an ongoing disease process [77].
The ATN system does not make assumptions about the causal relationships between the biomarkers [76]. This makes it compatible with the different hypotheses about the cause of AD. Although used here as a classification system for preclinical AD, The ATN system can also be used independently of the cognitive status or diagnosis of individuals [76]. The ATN system is designed to be dynamic, and if novel biomarkers are discovered they could be added to one of the three categories, or to a new category [77], and NfL has been discussed as new biomarker for the N-category.
The binary division into pathology groups could be viewed as a limitation. However, binary cutoffs exist in many diseases. It is also possible to modify the ATN system and incorporate an intermediate stage of pathology [76], but we have chosen the binary division since it is easy to use.

Although the ATN system is frequently used in research, it should be pointed out that it is not intended for clinical use [77]. More information about the ATN system and the cutoffs used here is given in the methods section.

1.9.2 CSF BIOMARKERS

A biomarker is an objective indicator of a biological process, that measures disease risk or prognosis, guides diagnosis or monitors therapeutics [64]. CSF surrounds the subarachnoid space and the ventricles of the brain. Since it is in contact with the brain it can reflect biochemical changes and is therefore a rich source of biomarkers related to neurodegeneration [64]. CSF can be extracted from the lumbar region between the vertebrae via lumbar puncture (LP), which is a commonly used procedure that is considered safe [29]. CSF biomarker concentrations are normally assessed using enzyme-linked immunosorbent assays (ELISA).

Pathological levels of tau and amyloid can be detected as early as 20-30 years before the onset of cognitive symptoms [3]. The core AD CSF biomarkers T-tau , P-tau and $A\beta_{42}$ have a high (80-90%) sensitivity and specificity for AD diagnosis [64]. Combining the biomarkers increases the accuracy of the diagnosis even further [64]. Some studies also investigate these biomarkers in plasma [29,92].

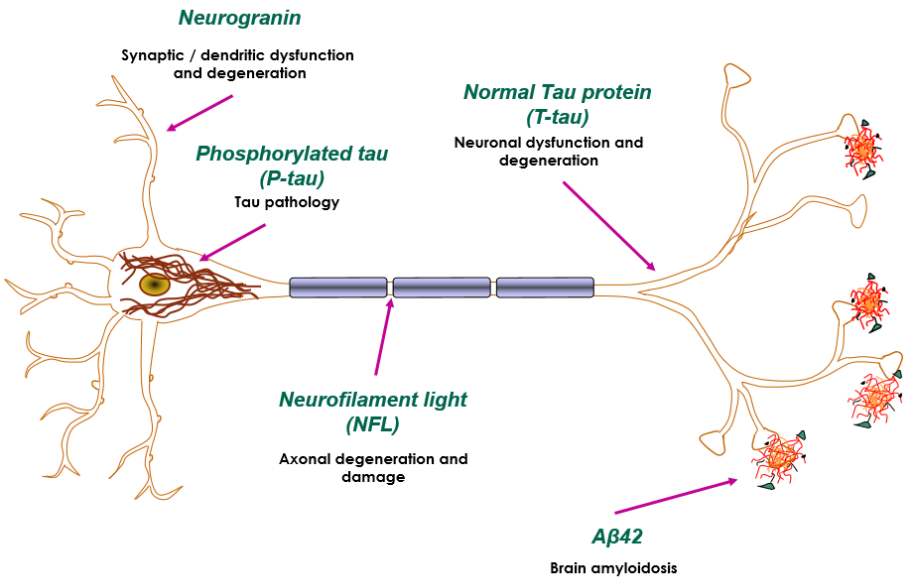

Figure 3. Schematic diagram of a neuron with Aβ depositions in the extracellular matrix, neurofibrillary tangles in the neuronal soma, neurofilament light in the axon and neurogranin in the dendrites. Image courtesy of Kaj Blennow, modified from Blennow & Zetterberg, Journal of Internal Medicine, 2018[93]

1.9.3 AMYLOID-β_{42} ($A\beta_{42}$)

$A\beta_{42}$ is a 42 amino acids long isoform of Aβ. It is the first biomarker to become abnormal according to Jacks model [76]. Low levels of $A\beta_{42}$ in CSF are considered pathological, and may reflect deposition of amyloid in the cerebral cortex in AD patients [3,29] (Figure 3). CSF $A\beta_{42}$ is 50% reduced compared to controls in AD [3,29]. Reduced CSF $A\beta_{42}$ levels differentiates AD from many other psychiatric and neurological conditions, although low CSF $A\beta_{42}$ can also be seen in a few other diseases [29].

1.9.4 PHOSPHO-TAU (P-TAU)

CSF P-tau is a biomarker for neurofibrillary tangles [94] (Figure 3). There are several variants of P-tau phosphorylated at different sites [94]. CSF P-tau levels are increased in MCI compared to healthy controls

and correlated with cognitive decline in MCI [95]. CSF P-tau levels are elevated in AD / probable AD compared to controls and to other diseases [92,96,97], and associated with burden of neurofibrillary tangles [98]. P-tau can discriminate AD from healthy controls as well as a few neurological conditions such as frontotemporal dementia with a high sensitivity and specificity [29,99]. CSF P-tau can also separate MCI patients from healthy controls, and predict progression from MCI to AD [29]. However, P-tau combined with T-tau or Aβ can differentiate AD from other dementias with higher specificity than P-tau alone [3,100].

1.9.5 TOTAL TAU (T-TAU)

T-tau reflects neuronal damage and is a general marker of neurodegeneration and damage to cortical neurons [3,99] (Figure 3). T-tau is elevated in the CSF of AD patients (300% compared to controls) [3,35], but also in some other conditions[92,96,101-103]. There are six isoforms of tau in the brain [32,40]. Most CSF tau exists in fragmented form [35,104]. Tau may be fragmented differently in different tauopathies [35],and the ELISA assay used in this project to measure T-tau is based on an antibody that captures all isoforms, regardless of phosphorylation or fragmentation [96].This leads to some overlap in the T-tau measure, since P-tau is a part of T-tau and the two biomarkers cannot be completely separated.

1.9.6 NEUROFILAMENT LIGHT (NFL)

Neurofilaments are structural cylindrical proteins solely expressed in neurons [105,106]. They occur in high concentrations particularly in the axon, but are also present in the dendrite and the soma [106] (Figure 3). Neurofilaments are candidate biomarkers of axon injury in several neurodegenerative, as well as vascular and inflammatory conditions [105]. Neurofilaments increase as a result of neuron damage in both CSF and serum in several neurological conditions [105,106]. There are 3 main types of neurofilaments, categorized based on their weight – light medium and heavy[105]. In this project we have studied neurofilament light (NfL) which is abundant in axons, especially in large caliber myelinated axons, and reflects damage to subcortical large-caliber myelinated axons [107-109]. Elevated levels of NfL have been reported in a number of neurodegenerative conditions [105,110-112] including AD and MCI[113] and can predict progression [114], and it seems to be an early biomarker [115].

1.9.7 NEUROGRANIN (NG)

Neurogranin (Ng) is a dendritic and post-synaptic protein[116] that reflects synaptic damage[117,118], and has been proposed as a potential novel AD biomarker (Figure 3). Synaptic loss is an early and important feature of neurodegenerative disorders including AD, but the mechanisms behind it remain unclear [119]. CSF Ng is increased early in AD [117,118,120,121] and expressed in brain regions affected in the disease [117]. Ng is also proposed to have a function in memory consolidation [117,122]. It binds to the calcium-signaling protein calmodulin which is involved in signaling processes important for memory formation [116,119]. Ng often occurs as fragments [122].

CSF Ng has been shown to predict cognitive decline in cognitively normal individuals [120]. In contrast to NfL, elevated CSF Ng is specific to AD and is not increased in other neurodegenerative conditions [118,123].

2 AIM

The overall aim of this thesis is to investigate cognition in older adults and its relation to biomarkers of AD and neurodegeneration.

Specific aims for the studies

Study I

The aim of this study was to investigate if cognitive performance in a sample of healthy 70-year-olds differed between individuals with and without preclinical AD.

Study II

The aim of study II was to investigate if elevated CSF-Neurofilament light protein and CSF-Neurogranin levels were associated with slight cognitive decline in cognitively healthy 70-year-olds from the general population.

Study III

The aim of study III was to characterize CSF-NfL and CSF-Ng levels in ATN groups in a population-based sample of cognitively unimpaired older adults.

3 METHODS AND STUDY DESIGN

3.1 STUDY SAMPLE – THE GOTHENBURG H70 BIRTH COHORT STUDIES

This thesis is based on samples from the 2014-2016 examinations of the Gothenburg Birth Cohort Studies (the H70 studies) in Gothenburg, Sweden. Details of the sample, the recruitment process and the examinations have been described previously [2,124]. Study participants were born 1944, and were systematically selected based on predefined birth dates (every 70-year old Gothenburg resident born 1944 on dates ending with 0, 2, 5 or 8 was invited to participate, comprising an eligible sample of 1839 individuals.) (Figure 4). The eligible sample comprised registered residents in Gothenburg (including parts of the municipalities of Ale, Kungsbacka, Kungälv, Lerum, Mölndal) irrespective of place of living (i.e. private household or residential care.) Information about birth dates and home addresses were obtained from the Swedish population registry [2]. Out of 1839, 172 individuals were excluded because they were deceased, could not be traced, could not speak Swedish, or were no longer living in Gothenburg, leaving 1667 to be contacted. Invitation letter (including study information and consent form) were sent by mail and followed up by a phone call (after up to three reminders had been sent). A total sample of 1203 individuals (72.2%, 559 men and 644 women) participated in the study (mean age 70.5 years [2]). Out of 1203, 430 consented to lumbar puncture (LP) whereof 108 individuals had contraindications for LP (immune modulated therapy, anticoagulant therapy and cancer therapy) and were excluded. The remaining 322 individuals took part in LP (response rate: 35.7%). Individuals with dementia (n=6) were excluded and a Clinical Dementia Rating (CDR)[19] score was assessed by a neurologist. 259 individuals had a CDR score of 0 and 57 individuals had a CDR score of 0.5 and were used as sample in study I. Of these, we selected 256 individuals with NfL data and 258 with Ng data for study II and III (Figure 5).

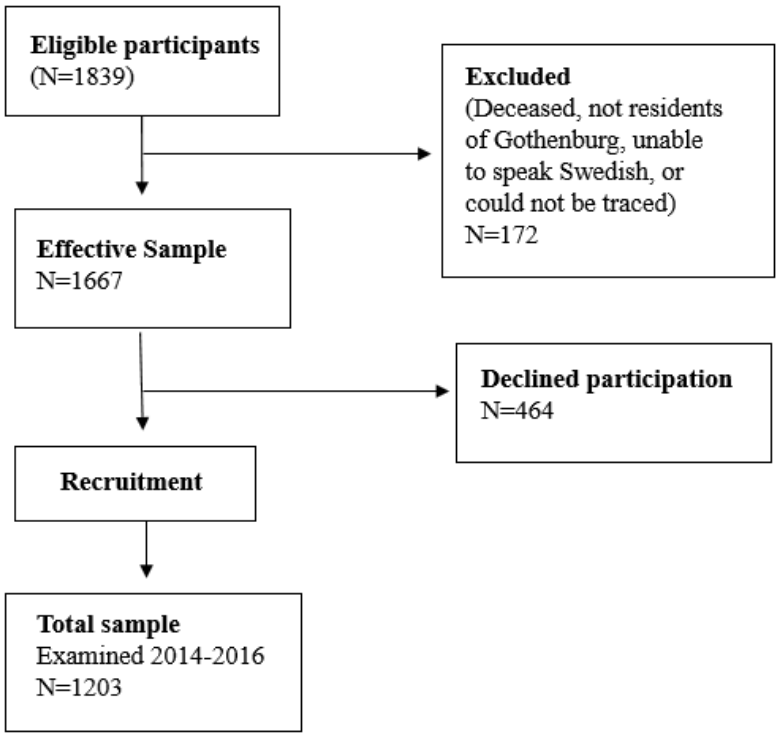

Figure 4. Flowchart of the sample recruitment process of the 2014-2016 examinations of the Gothenburg birth cohort studies. The flowchart was modified by the author and has been published previously [2] .

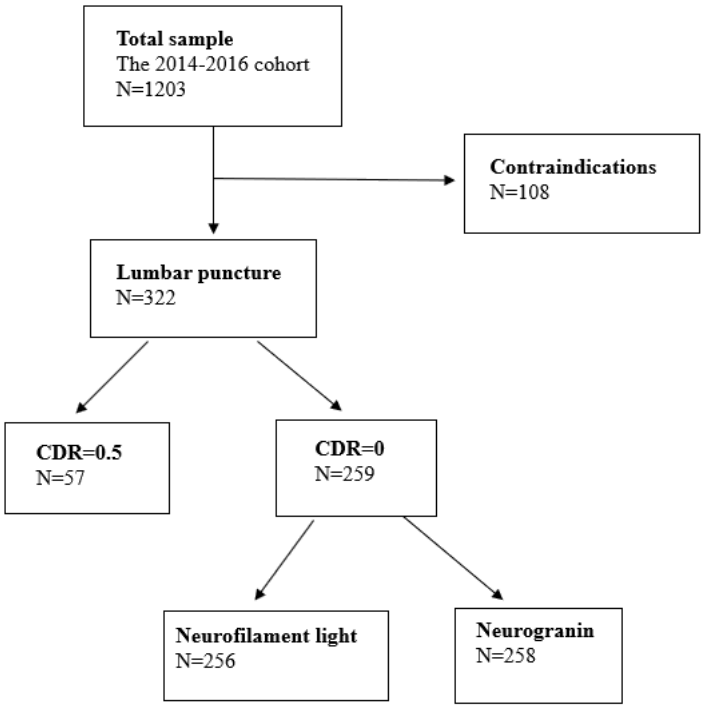

Figure 5: Flowchart of the samples used in this thesis.

3.1.1 THE 1944 COHORT

The cohort of 70-year-olds born 1944 (n=1203) was examined during 2014-2016. This cohort is the largest and most comprehensively studied H70 cohort thus far[2]. The sample consisted of 559 men and 644 women. These individuals had a high education (almost 30% had a university degree) with 13 mean years of education and a high mean MMSE score (28.8), which indicates a high cognitive ability. Approximately one fifth were still working (part or full time or periodically), 65.8% reported daily internet use, and a small number (2.1%) were residing in sheltered living. These characteristics imply that the 1944 cohort is a highly functioning sample socially and cognitively [2]. Around 90% had children, around 70% had a partner,

around 9 % were smokers and the majority (84.5%) were born in Sweden [2].

3.2 GENERAL EXAMINATION AND NEUROPSYCHIATRIC ASSESSMENTS

Participants took part in a one-day general examination (including breakfast and lunch) at the Psychiatry Cognition and Old Age Psychiatry clinic at Sahlgrenska University Hospital in Gothenburg, Sweden, (formerly Neuropsychiatric outpatient clinic) or in their homes. Participants were examined by experienced psychiatric research nurses. Somatic examinations included spirometry, genetics and family history, medication use, physical examination, and ECG. The neuropsychiatric examinations consisted of ratings of psychiatric symptoms, cognitive and memory tests, and personality changes [2,124]. A psychologist or research nurse performed key informant interviews [124]. Comprehensive somatic examinations were also performed, comprising blood samples for genetic analyses, genotyping, blood pressure, lung function, ophthalmological and audiological examination, body composition, medications and self-rating questionnaires [2]. Several additional examinations were also performed, including dietary examination, brain imaging, LP and focus groups / qualitative studies. History of stroke and transient ischemic attack (TIA) and years of education were acquired from self-reports and close informants. Depression was diagnosed according to DSM-5 [125]. Dementia was diagnosed according to criteria from the Diagnostic and Statistical Manual of Mental Disorders DSM III-R [126] based on information from the cognitive examination and from key informant interviews. The DSM-III-R [127] has been used in the Gothenburg Birth Cohort studies since they started in the 70s.

3.2.1 COGNITIVE EXAMINATION

The cognitive examination, which study 1 and 2 were based on, (performed by a psychologist, research nurse, psychiatrist or medical doctor) include tests from the Dureman and Sälde test battery, that was frequently used in Sweden during the 1970s when the H70 studies started, and hence allows for comparability over time [128,129]. The cognitive and psychological examinations also included other tests and assessments such as tests of short- and long term memory, construction apraxia (copying figures), finger agnosia (naming figures), orientation, abstract thinking (understanding proverbs), ideational apraxia (following instructions), naming the last two prime ministers, as well as a brief interview about cognitive activities and memory [2]. These assessments are used as support for dementia diagnosis, and for rating other neuropsychiatric conditions. A detailed description of the neuropsychological test battery used in this thesis is described below.

3.2.2 MEMORY TESTS

Immediate and delayed recall was assessed with a 12 object recall test where the participants were shown 12 objects, which were then immediately recalled (Immediate recall) versus repeated after 5 minutes (Delayed recall). The Supra span test comprised a list of 10 clothing items that the participant should immediately repeat in any given order (free recall) [130].

Word memory is a 10 word recall test. The participants were asked to read a list of words aloud without receiving instructions to memorize the list. The participant was then told to repeat the words in free order. For non-verbal memory, a version of the Thurstone's picture memory test which consisted of a list of 28 images was presented to the test person. After the presentation of images, the participant was shown a set of 4 similar images in which the task was to identify the correct one [129,131]. For all memory tests, the total score was the total number of recalled words/objects [129].

3.2.3 LANGUAGE TESTS

Tests of language included a test on semantic memory (Word fluency) and a test on phonetic fluency (FAS)[132]. In Word fluency the test person was asked to generate as many animal names as possible during one minute (one score is given for each animal named). In FAS, the participant was asked to generate as many words as possible starting with the letters F, A and S, during one minute for each letter. The score was the total score for all the letters.

3.2.4 EXECUTIVE FUNCTION AND MENTAL SPEED TESTS

SRB2 is an 8-minute figure logic test [129] that measures inductive reasoning ability [129]. The test comprises 30 logical problems, and in each series, there were 5 figures out of which 4 had common features. The task was to discover which figure deviated from the other three figures[128]. The score was the sum of the number of correct items with a maximum score of 30 points [129].

SRB3 is a block design test [128,129], where the participant is tasked to replicate a pattern of colored blocks. It measures perceptual speed and spatial ability and may also (in combination with other tests) function as an estimate of intellectual ability [128,129]. Each test should be completed as quickly as possible and the maximum score was 42 points [129].

Psif is a test of mental speed based on figure identification. In the test, there are 60 items, including 6 figures per row and the task is to identify the identical figure among the other ones. Total score (maximum score is 60) is the number of correct tasks performed during 4 minutes minus the number of wrong items. The total score is then divided by four in order to penalize for guessing and wrong answers[129] [128].

Digit span backwards is an executive function / working memory test in which the test person memorizes a series of digits in reverse order. There are 8 separate tasks, and the number of digits increases for each level of difficulty.

3.2.5 MINI MENTAL STATE EXAMINATION AND CLINICAL DEMENTIA RATING

Global cognitive status was assessed by Mini Mental State Examination (MMSE) [133] and CDR [19,134,135]. MMSE is a quick test including orientation, learning, simple arithmetic, following simple instructions and drawing overlapping figures that is frequently used in clinical routine for screening of dementia [133]. The test provides a rough estimate of the global cognitive ability. A Swedish version of the MMSE was used.

CDR is a scale consisting of a composite score from the cognitive domains memory, orientation, judgement and problem solving, community affairs, home and hobbies, and personal care [134]. The scores in these domains are obtained from semi-structured interviews. The CDR scale is widely used in research and in the clinic to assess cognitive function by assigning a score ranging from 0 (no impairment), 0.5 (questionable impairment), 1 (mild impairment), 2 (moderate impairment) to 3 (severe impairment) [134]. The CDR scores were assigned by the research nurses and a neurologist, using an algorithm. CDR can be assigned by two methods, the CDR sum of boxes, and the global CDR, which has been used here, and these methods compare well with each other for dementia staging [136].

3.2.6 CEREBROSPINAL FLUID SAMPLING, GENOTYPING AND BIOMARKER ANALYSES

Prior to LP, participants (n=322) were investigated for contraindications. They underwent brain computed tomography (CT) or magnetic resonance imaging (MRI) to detect increased intracranial pressure. Other contraindications included immune-modulated therapy, cancer therapy and anticoagulant drugs such as warfarin. As previously described, [124] LPs were conducted in the morning, according to the Sahlgrenska University Hospital routine by an experienced neurologist/physician. CSF was collected from the L3/L4 or L4/L5 interspace and an atraumatic needle with 22-25 gauge was used. The first 10 mL of CSF were collected in a polypropylene tube and centrifuged at 1800 RCF at 20˚C for 10 min [2]. The supernatant was gently mixed and aliquoted in polypropylene tubes and stored at –70˚C

[2,124]. The CSF samples were transported to the laboratory (Clinical Neurochemistry laboratory, Sahlgrenska University Hospital/ Mölndal) immediately after collection, as previously described[124].
The following assays were used to quantify the biomarkers: a sandwich ELISA (INNOTEST® htau Ag and PHOSPHO_TAU (181P), Fujirebio (formerly Innogenetics) was used to measure CSF T-tau and P-Tau (phosphorylated at threonine 181) [96,137]. A sandwich ELISA (INNOTEST® β-amyloid$_{1-42}$) was used to measure CSF Aβ-$_{42}$ (Aβ starting at amino acid 1 and ending at amino acid 42) [138].
T-tau, P-tau and Aβ-$_{42}$ were analyzed as part of clinical routine diagnostics at the Mölndal Clinical Neurochemistry Laboratory. CSF-NfL and Ng were stored in -80°C. NfL and Ng concentrations were measured using in-house ELISA assays developed at the Mölndal Clinical Neurochemistry Laboratory. These methods have been described previously [117,139,140].
Briefly, the single-nucleotide polymorphisms rs7412 and rs429358 in *APOE* were genotyped, as described before [124], and this genotype data was used to define the *APOE* alleles. *APOE* genotype data was missing for 5 individuals. *APOE ε4* carriers were defined as homo- or heterozygotes.

3.2.6.1 ATN CLASSIFICATION AND BIOMARKER CUT-OFFS

The ATN system is a research framework used for diagnosing preclinical AD in studies, developed by Jack et al [76]. This system consists of 8 possible binary categories where A denotes amyloid, T denotes P-tau and N denotes neurodegeneration. Pathology is indicated by + and non-pathology by -, and can be measured by CSF, PET or MRI. Each participant was classified according to this scheme and grouped into one of the 8 biomarker categories. We defined pathology as follows: amyloid pathology (A+): CSF Aβ$_{42}$ concentration ≤ 530 pg/mL. Phospho-tau pathology (T+): CSF P-tau ≥ 80 pg/mL and neurodegeneration (N+): CSF T-tau ≥ 350 pg/mL [124,141]. These cutpoints for AD biomarker pathology were chosen since they have been used in our studies previously [142].

3.3 ETHICAL CONSIDERATIONS

The study was performed according to the declaration of Helsinki and was approved by the Regional Ethical Review Board in Gothenburg. All participants and/or their close relatives were informed about the study proceedings and asked to sign a consent form. The ethical permit approval numbers are as follows: 869-13 for the H70 study, and 006-14 and T-703-14 for the collection of CSF biomarkers [2].

3.4 STATISTICAL ANALYSES

Study I

Student's t-test was used to determine differences in means, and Fischer's exact test was used for proportions. When variances were not equal (according to Levene's test) Satterthwaite's t-test was used instead of Student's t-test. We also used the Hochberg method for correction for multiple testing. Correlations were determined using Spearman correlation. Post-hoc power analyses were performed in Stata v.14.0, StataCorp, Texas, USA. An α-value of 0.05 and a power value of 80% were used to investigate the required sample size (using mean values and standard deviations from the observed sample). Different effect sizes were tested; based on both significant findings and negative results.

Study II

Differences in means of cognitive tests in individuals with high vs low NfL and Ng levels (based on the median) were determined using Student's or Satterthwaite's t-test. Characteristics were tested using Fischer's exact test. The NfL and Ng variables were skewed, so these variables were log-transformed. We also divided the NfL and Ng variables into tertiles, compared the tertiles in t-tests, and performed a Whitney Mann U-test as a sensitivity analysis. We also performed post-hoch power analyses and Hochberg correction for multiple testing.

Study III

In this study we examined the levels of (log)NfL and (log)Ng in the different ATN groups. We used Student's or Satterthwaite's t-test and also performed a univariate analysis of variance (ANOVA) and ANCOVA analysis with the covariates age, sex, stroke and *APOE ε4* carriership. To test differences in means of NfL and Ng in ATN groups the ATN groups were also compared pairwise. We also examined correlations between the biomarkers NfL, Ng, T-tau, P-tau and Aβ using Spearman correlations.
Chi square test was used to compare *APOE* ε4 status in NfL and Ng, divided into tertiles, and t-test were used to compare NfL and Ng levels in amyloid and tau pathology groups.

All analyses in these studies were carried out using IBM SPSS Statistics for Windows (v. 25) and Stata (v.14.0). Statistical significance was determined as $p<0.05$.
Normal distribution in all studies was assessed using a combination of graphical assessment (histograms, stem and leaf plots and quantile-quantile plots) and the Shapiro Wilk test. In cases with slightly skewed data, t-tests were used since the sample size was relatively large and a slight skew is not likely to have an impact on the reliability of the results in large samples. In cases where the skew was moderate to large, we log-transformed the variables.

4 RESULTS

4.1 STUDY I

In this paper we investigated the cognitive performance of older adults (mean age=70.6) with normal cognition (defined as CDR0, n=259) in several cognitive tests. We hypothesized that there would be slight differences among people with AD biomarkers compared to those without. We could show that, in most cognitive tests in our battery, the cognitive performance was similar in people with and without biomarkers, except in the memory tests Supra span (p=0.003), Delayed recall (p=0.042) and Immediate recall (p=0.033), where participants with A-T-N+ performed worse. We could also show that among participants with CDR0.5 (n=57) those with amyloid pathology had worse score in the language test category fluency (P=0.003).

Cognitive test performance in 70-year olds from the Gothenburg Birth Cohort Studies (n=259) with Clinical Dementia Rating 0 according to the ATN classification system for preclinical Alzheimer's disease

	Normal CSF (A-T-N-) N = 138	A-T-N- Mean Score (SD)	A+T-N- N = 34	A+T-N- Mean Score (SD)	p^1	A+T-N+ N = 20	A+T-N+ Mean Score (SD)	p^2	A+T+N+ N = 6	A+T+N+ Mean Score (SD)	p^3	A-T-N+ N = 49	A-T-N+ Mean Score (SD)	p^4	A-T+N+ N = 12	A-T+N+ Mean Score (SD)	p^5
MMSE	138	**29.28(0.90)**	34	29.35(0.77)	0.677	20	29.15(0.93)	0.542	6	**28.50(1.23)**	**0.043**[*†]	48	29.25(1.14)	0.841	12	29.17(0.94)	0.672
Memory																	
Immediate recall	138	**8.38(1.67)**	34	8.06(1.46)	0.299	20	8.10(1.92)	0.487	6	8.67(0.82)	0.682	48	**7.79(1.56)**	**0.033***	12	8.17(1.85)	0.669
Delayed recall	138	**7.91(1.72)**	34	7.53(1.75)	0.256	20	7.20(2.04)	0.096	6	8.67 (1.03)	0.285	48	**7.33(1.51)**	**0.042***	12	7.58 (1.98)	0.539
Word memory	137	5.73 (1.86)	33	5.18 (1.88)	0.131	20	5.50 (1.67)	0.601	6	6.00(2.19)	0.730	47	5.55 (1.64)	0.563	11	5.27 (1.56)	0.429
Supra span (BUSII)	132	**7.82 (1.42)**	32	7.56 (1.74)	0.384	18	7.89 (1.23)	0.841	5	8.00 (1.23)	0.778	46	**7.07 (1.64)**	**0.003**	12	7.92 (1.24)	0.817
Thurstone's picture memory test	124	23.06 (3.59)	32	22.09(4.17)	0.193	18	21.89(5.96)	0.428	6	20.50 (6.32)	0.369	46	22.76 (3.06)	0.621	12	24.08(3.23)	0.341
Language																	
Word fluency	137	**25.33(6.73)**	34	25.85(6.11)	0.678	20	23.30(7.24)	0.215	6	**30.33(2.58)**	**0.003**	48	23.35(5.54)	0.070	12	26.67(6.07)	0.506
FAS	132	42.27(13.56)	33	42.33(13.69)	0.982	19	42.84(12.64)	0.863	5	40.00(11.09)	0.712	46	40.33(15.32)	0.419	11	42.55(14.71)	0.949
Executive function																	
SRB2	134	20.56(4.28)	34	20.53(3.87)	0.970	19	20.68(4.57)	0.907	6	20.17(2.48)	0.824	48	20.25(3.78)	0.659	12	21.17(4.11)	0.638
Digit span backwards	136	4.63(1.19)	34	4.41(0.96)	0.333	19	4.95(1.27)	0.273	5	4.80(0.45)	0.743	47	4.34(1.05)	0.146	12	4.67(1.07)	0.907
Visuospatial																	
SRB3	133	22.11(6.64)	33	23.09(5.78)	0.436	19	21.74(8.39)	0.827	5	22.20(7.33)	0.975	47	20.30(6.62)	0.110	12	22.75(8.66)	0.754
Mental speed																	
Psif	136	29.90(7.42)	33	29.67(7.63)	0.870	19	27.95(10.41)	0.309	6	29.83(7.99)	0.982	48	29.88(7.95)	0.982	12	30.00(4.86)	0.965

p^1 = difference in mean score for A+T-N- compared to normal CSF with *T*-test. p^2 = difference in mean score for A+T-N+compared to normal CSF with *T*-test. p^3 = difference in mean score for A+T+N+ compared to normal CSF with *T*-test. p^4 = difference in mean score for A-T-N+ compared to normal CSF with *T*-test. p^5 = difference in mean score for A-T+N+ compared to normal CSF with *T*-test. [†]Previously published in Kern et al. [6]. There were no participants with CDR 0 in the groups A-T+N- and A+T+N-. *Not significant at the 5% level after Benjamini-Hochberg correction

Figure 6. Cognitive performance in 70-year olds with CDR0 stratisfied by ATN class from Study I. Reprinted from Journal of Alzheimer's Disease,Vol 79, Maya Arvidsson Rådestig, Johan Skoog, Henrik Zetterberg, Jürgen Kern, Anna Zettergren, Simona Sacuiu, Margda Waern, Hanna Wetterberg, Kaj Blennow, Ingmar Skoog, Silke Kern, Cognitive Performance and Cerebrospinal Fluid Markers in Preclinical Alzheimer's Disease: Results from the Gothenburg

H70 Birth Cohort Studies, Pages No., 225–235 Copyright (2021), with permission from IOS Press. The publication is available at IOS Press through http://dx.doi.org/[doi: 10.3233/JAD-200751]

Cognitive test performance and cerebrospinal fluid biomarker status in 70-year olds from the Gothenburg Birth Cohort studies (n=57) with Clinical Dementia Rating 0.5

	Amyloid pathology N = 13	Amyloid pathology mean score (SD)	No amyloid pathology mean score (SD)	p^1	T-tau pathology N = 18	T-tau pathology mean score (SD)	No T-tau pathology mean score (SD)	p^2
MMSE	13	27.08 (1.38)	27.77 (1.51)	0.142	18	27.33 (1.19)	27.74 (1.62)	0.341
Memory								
Immediate recall	12	6.67 (2.23)	6.51 (1.50)	0.778	17	6.06 (1.75)	6.76 (1.60)	0.149
Delayed recall	12	6.67 (1.92)	5.67 (1.89)	0.114	17	5.65 (1.80)	6.00 (1.99)	0.534
Word memory	12	3.58 (1.31)	3.71 (1.57)	0.793	17	3.35 (1.22)	3.84 (1.61)	0.275
Supra span (BUSII)	10	6.90 (1.45)	7.03 (1.70)	0.832	14	7.43 (1.02)	6.83 (1.81)	0.150
Thurstone's picture memory test	10	20.20 (3.05)	20.07 (4.46)	0.933	16	20.94 (3.96)	19.71 (4.30)	0.339
Language								
Word fluency	13	**17.38 (5.77)**	**22.95 (5.54)**	**0.003**	18	20.44 (7.03)	22.22 (5.50)	0.311
FAS	7	27.14 (7.20)	33.03 (10.94)	0.179	15	30.60 (9.61)	32.88 (11.12)	0.499
Executive function								
SRB2	12	18.42 (3.55)	18.44 (3.55)	0.985	16	19.56(3.25)	17.95 (3.56)	0.126
Digit span backwards	12	3.42 (0.90)	3.93 (1.01)	0.117	17	3.53 (0.72)	3.95 (1.09)	0.155
Visuospatial								
SRB3	11	15.45 (7.46)	17.24 (7.66)	0.493	16	18.38 (7.19)	16.22 (7.75)	0.346
Mental speed								
Psif	12	20.58 (5.21)	22.98 (7.26)	0.293	17	23.35 (8.49)	22.03 (6.09)	0.516

p^1 = difference in mean score for amyloid pathology compared to non-amyloid pathology with *T*-test. p^2 = difference in mean score for T-tau pathology compared to non-T-tau pathology with *T*-test. There were two participants with P-tau pathology, and this group was excluded from the analyses due to the small size.

Figure 7. Cognitive performance in 70-year olds with CDR0 stratisfied by pathology from Study I. Reprinted from Journal of Alzheimer's Disease,Vol 79, Maya Arvidsson Rådestig, Johan Skoog, Henrik Zetterberg, Jürgen Kern, Anna Zettergren, Simona Sacuiu, Margda Waern, Hanna Wetterberg, Kaj Blennow, Ingmar Skoog, Silke Kern, Cognitive Performance and Cerebrospinal Fluid Markers in Preclinical Alzheimer's Disease: Results from the Gothenburg H70 Birth Cohort Studies, Pages No., 225–235 Copyright (2021), with permission from IOS Press. The publication is available at IOS Press through http://dx.doi.org/[doi: 10.3233/JAD-200751]

4.2 STUDY II

In this study we investigated the cognitive performance in older adults (mean age =70.6) with increased levels of neurodegeneration related biomarkers, NfL and Ng (CDR=0, n=258). We hypothesized that individuals with elevated CSF-NfL and CSF-Ng would display slightly worse scores on neurocognitive tests. We divided the biomarker variables based on the median and also divided them into tertiles. We found that individuals with high NfL performed worse than those with

low NfL in memory and language, and participants with high Ng performed worse than those with low Ng in memory, although in most tests there were no differences between participants with high and low levels of the biomarkers. When stratified according to amyloid and tau pathology, we could show that those with high NfL and tau pathology performed worse in memory than those without tau pathology. Individuals with high NfL and amyloid performed worse in memory than those without amyloid pathology. Regarding participants with high Ng, those with amyloid pathology had lower score on global cognition (MMSE).

4.3 STUDY III

In this paper we investigated the CSF-NfL and CSF-Ng concentrations in the different ATN groups with t-tests in older adults (mean age= 70.6). We also performed an ANOVA and ANCOVA with pairwise comparisons. We found that participants with N+ had higher levels of NfL and Ng than N- in a t-test. The pairwise comparisons in the ANCOVA also showed a significant difference between the ATN groups in levels of Ng, but not for NfL. We found no difference in NfL or Ng levels in A+ compared to A-.

5 DISCUSSION

5.1 INTERPRETATION OF THE MAIN FINDINGS

In studies I and II, we found that the performance on most of the neurocognitive tests was similar in groups with and without amyloid and tau pathology. This contradicts many other studies in non-demented individuals that have found subtle differences in baseline cognition or in longitudinal cognitive decline between individuals with amyloid and/ or tau pathology and those without [13,143-147]. There are many ways to interpret these mostly negative results. They could be seen as an argument that there is not much evidence for any cognitive decline in the preclinical phase of AD. The reason why most of our results were negative in study I and Study II could depend on the fact that our participants were examined in a very early phase and were relatively healthy, making it unlikely to detect cognitive differences. The cognitive battery used here may not have been suitable to detect cognitive decline of a subtle character. There are also statistical reasons why we may have been unable to detect more differences between groups, such as small sample size (*i.e.* low power). Other groups have performed similar studies and failed to detect differences in cognitive tests in cognitively normal older adults with and without AD biomarkers [83,85]. However, the findings of slight differences between individuals with and without AD pathology in some cognitive tests are interesting, and therefore the rest of the discussion will focus on interpreting the positive results.

5.2 SUBTLE COGNITIVE DECLINE CAN BE SEEN IN GROUPS WITH NEURODEGENERATION

In study I, we found a few subtle cognitive differences, especially in individuals with CDR0 who had biomarkers related to neurodegeneration. We showed that the group with only T-tau pathology (A-T-N+) had a slight decline in several memory tests (Delayed recall, Supra span, immediate recall) compared to A-T-N- (Figure 6). The group with A-T-N+ is also known as suspected non-AD pathophysiology (SNAP) according to the Preclinical Working

Group of the National Institute on Aging and Alzheimer's Association (NIA-AA) criteria published 2011[81]. Participants with SNAP have a heterogenous disease profile, which may progress to other dementias than AD. However, it has been reported that some progress to AD even though they do not display amyloid pathology [148]. Results from our study are in line with another study in community dwelling volunteers [78], in which individuals with SNAP (defined in their study as having abnormal T-tau or P-tau in the presence of normal $A\beta_{42}$) performed worse on an episodic memory test than stage 1 and the normal group. The stage 3 group (defined in their study as abnormal Aβ, T-tau /P-tau and slight memory impairment) according to the NIA-AA criteria 2011.), [78] also performed worse on MMSE compared to stage 1 and the normal group which is also in line with our A+T+N+ group that performed low in MMSE compared to A-T-N-[78]. Our results from Study I suggest a possible relationship between neurodegeneration (T-tau) or cognitive deficits in otherwise cognitively healthy participants, who may progress to AD. Our results from study II also emphasize the possible relationship between neurodegeneration and slight cognitive decline. It is difficult to know if the slight cognitive decline we have seen is associated with preclinical AD or if it is due to normal aging related memory decline in those with high levels of NfL and Ng, but the stratification according to amyloid and tau pathology in which the combination of amyloid and tau pathology and high levels of NfL and Ng showed a slight cognitive decline, suggests that there could be a connection to preclinical AD. Our result of slightly poorer memory performance in those with high Ng levels is in line with a study that also found a connection between Ng and memory in cognitively healthy older adults [149]. However, in this study, Ng was related to memory independently of the AD biomarkers CSF P-tau T-tau and $A\beta_{42}$, whereas in our study there was an association between Ng in combination with amyloid pathology and worse scores in MMSE. NfL and T-tau are both biomarkers of neurodegeneration. An interesting and debated question is if tau could be a driving factor in AD pathogenesis. In our assay, P-tau is a part of T-tau, so the elevated T-tau levels in N+ may to some extent reflect individuals with neurofibrillary tangles although the number of such individuals is low in our sample. T-tau primarily reflects neurodegeneration regardless of the cause or disease (it is not AD specific) and neurofibrillary tangles seem to mediate cognitive decline. However, our results could imply

that there might be a link between neurodegeneration, regardless of its cause, and cognitive decline.
In conclusion, there may be some support in our studies for the notion that early neurodegeneration in the form of axonal and synaptic damage (T-tau, NfL and Ng biomarkers) could lead to a slightly lower cognition even in otherwise cognitively healthy persons, since neurodegeneration markers seem to be the common denominator for many of our participants with lower cognitive scores. It is, however, not clear whether it is related to AD pathology or neurodegeneration due to other reasons such as normal aging, and needs to be investigated further.

5.3 HOW DOES NEURODEGENERATION RELATE TO COGNITION?

A possible explanation why increased Ng and T-tau might be associated with a slight cognitive impairment could be that synaptic dysfunction or loss may be a link between neurodegeneration and cognition. Synapses play a role in cognitive functions such as memory and learning [150] and synapse loss and dysfunction is a characteristic symptom in AD and many other neurodegenerative diseases [119,151]. AD has therefore been called a "synaptopathy" [119,151]. Neuronal plasticity is important in the healthy brain, and the mechanisms of plasticity may be disrupted, even in early AD [151]. This disruption of neuroplasticity could be driven by amyloid and tau pathology and may lead to a loss of synapses [151]. Synaptic dysfunction and loss has been seen in early AD already at the MCI stage [36,152-154] and is especially evident in the hippocampus, which is involved in memory functions [155].Synaptic changes also occur in the hippocampus and prefrontal cortex during normal aging, as has been shown in monkey studies [156], and has been suggested as a cause of aging related cognitive decline [156].
The mechanisms behind synaptic loss in AD and how synaptic dysfunction and loss relates to cognition are not fully understood [152] but it has been suggested as a mechanism linking pathology to disease symptoms [151,152]. Synaptic pathology has often been described as the best correlate of cognitive impairment in AD [153,154] and has been shown to correlate more strongly with dementia symptoms than amyloid and tau pathology[157,158].Synapse loss has been shown in some studies to affect or correlate to cognition [36] including the tests MMSE

and memory (delayed recall) [152,159] which is in line with our result of worse score in MMSE and memory in individuals with high levels of Ng in Study II.
Increased levels of Ng have been linked to accelerated cognitive decline and poorer memory performance [117,149]. Ng is also a calmodulin-binding protein involved in calcium signaling and long term potentiation [116,160] and animal research has shown that knocking down Ng leads to inhibition of long term potentiation and cognition, while upregulating it improves cognition [149]. Another possibility could be that Ng has important functions in synapses and that the loss of the proteins normal function leads to cognitive symptoms [149].
There are also other factors that may underlie cognitive decline in AD, for example neuronal death, which is also a prominent and early feature of the disease [30,161]. The role of neuronal death in causing cognitive symptoms has been discussed. It correlates with AD symptoms [161]. However, it might not be as likely to act as a mediator of cognitive symptoms as synaptic loss, since synaptic loss occurs earlier and to a greater extent and correlates more strongly with cognitive symptoms [162]. The brain can also compensate for loss of neurons [50]. Mice studies also show that neuronal loss leads to cognitive decline, but this impairment can be reversed [50]. In addition to synapse loss and dysfunction, spreading of pathology via synaptic junctions may also play a role in AD [48].
In summary, synaptic loss or dysfunction might be an early pathologic process in preclinical AD or in aging related neurodegeneration independently of AD [149].

5.4 POSSIBLE CAUSES OF SYNAPSE PATHOLOGY IN AD

5.4.1 PLAQUES AND AMYLOID-β

There is plenty of evidence that deposits of fibrillar Aβ (plaques) have a role in contributing to the pathogenesis of AD [50]. The original amyloid cascade hypothesis postulates that amyloid is central in the AD disease progression as a driving factor in AD etiology [45]. However, this hypothesis has also been the subject of considerable debate [47]. It is

not clear whether the role of plaques is primarily to start a neurodegenerative cascade, mostly driven by other downstream mediators, or if the plaques themselves are neurotoxic or how they relate to the symptoms in AD [47,48,50]. The assumption that fibrils of amyloid are pathogenic is based on a number of observations, including the fact that Aβ occurs in plaques, is deposited earlier than tangles are formed, and has a cytotoxic effect in *in vitro* studies, and that genetic mutations in familial AD increase Aβ production.[30,51] Aβ is associated with and may induce neuronal changes [48,50,163] and rat studies have also demonstrated a toxic effect of Aβ [164]. Aβ pathology can also decrease the number of synapses [153].

Although the amyloid hypothesis is strongly supported by evidence in rare autosomal dominant AD, it is not clear how strongly it is supported for late onset sporadic AD [47]. There are also other hypotheses that could explain observations mentioned above, including a hypothesis stating that amyloid may be a protective response to neurodegeneration rather than a causing agent [51]. Although controversial, this hypothesis could explain why people with a lot of amyloid in the brain have little or no cognitive symptoms[51], as studies have shown a poor correlation between cognitive decline and Aβ deposition[30,39,163]; *e.g.* there are individuals with normal cognition who can tolerate a substantial burden of amyloid deposits without developing cognitive symptoms [28,165,166]. Amyloid depositions also occur in different regions than neuron loss (such as the frontal lobes) whereas tau pathology occurs in the same regions as neuron death (the entorhinal cortex and hippocampus) [47] and synapse loss [38,155]. Further challenges to the amyloid-cascade hypothesis include that many clinical trials with monoclonal antibodies targeting Aβ have failed as therapeutics may be directed against the wrong target [167]. Lately, there has been an increasing attention to the idea that an imbalance in the production and clearance of Aβ acts as a trigger of other downstream mediators of neurodegeneration, (a modification of the original hypothesis) [47]. These potential, mediators include tau and oligomers of Aβ and tau as discussed below.

Regarding synapse pathology, not much is known about the involvement of Aβ and its possible relationship to synaptic pathology in AD [155], but there does not seem to be much evidence of such a relationship [168]. One post mortem histopathological examination of AD brains showed that there were no correlation between levels of a

synaptic marker and plaques [157]. Another study in AD brains with immunolabeled synaptic biomarkers synaptophysin found no relation between the localization of synaptic pathology with plaques [169]. Another study with synaptophysin in AD brains found no correlation between synaptophysin level and plaques [170], and one study showed that loss of synaptic inputs can occur independently of Aβ deposition[155,171].

There seems to be no strong association between synapse loss and amyloid deposition in AD [151]. However, we saw a connection between MMSE score and amyloid in individuals with synapse pathology (high Ng) in Study II, and in study I we saw a relationship between amyloid pathology and worse score in the Word fluency test in participants with CDR0.5.

In conclusion, it is difficult to know if Aβ in fibrillar form has a role as a driving factor in the neurodegeneration seen in AD, or whether it is a cause of synapse loss in AD [47,155].

5.4.2 TAU AND NEUROFIBRILLARY TANGLES

Growing evidence suggest that tau may be involved in synaptic dysfunction and degeneration in AD [36,166]. Although the main function of tau is to stabilize axons, it has also been found in other parts of brain cells of AD patients, including synapses [36,48,151,155] and axons in the brains of healthy individuals. Although, the role of tau in synapses and synaptic dysfunction is still unclear [36,48], tau has been suggested as a mediator of synaptic damage in AD [155]. Some reasons for this include the correlation between cognitive decline in AD and the progression of tau pathology in the brain, the observation that synapse loss occurs in the same regions as neurofibrillary tangles, and that lower number of synaptic proteins in AD are associated with higher numbers of tangles [155]. However, it is not clear whether neurofibrillary tangles are required for causing neurodegeneration. [40] Some studies have shown a relationship between neurofibrillary tangles and synaptic or neuronal loss, and that tau pathology correlates better with neuronal loss than Aβ [47,48]. But the results have been mixed, and some studies have found a weak or no relationship between tangles and synaptic pathology in AD [157,169,170].

There are mixed results regarding the relationship between neurofibrillary tangles and cognition. Like amyloid deposits, neurofibrillary tangles can exist in the brain without dementia

symptoms [48]. However, many studies have shown that tangles correlate with cognitive decline [38,48,155] for example one study that found a correlation between neurofibrillary tangle count and MMSE [39]. And another study found an association between MMSE and some fragments of tau [104].
In study III we investigated the relationship between ATN groups and levels of neurodegeneration (NfL) and synapse loss (Ng) and found that participants with T-tau pathology (N+) had increased axonal and synaptic damage, but we could see no such relationship with amyloid pathology. This is not surprising since T-tau and NfL both reflect neurodegeneration, and if there is a correlation between tau and synapse loss, higher levels of Ng would be expected in those with tau pathology / neurodegeneration.

5.4.3 AMYLOID AND TAU OLIGOMERS AND SYNAPTIC DYSFUNCTION

It is not clear what causes cognitive decline in AD, but one explanation could be synapse dysfunction or loss, possibly mediated by oligomers of amyloid and tau[172].Oligomers such as dimers and monomers of both amyloid and tau are present in the brain. They are neurotoxic [28,48,55] and increased in AD [55]. Oligomers bind to neurons and may disrupt synapses [55] or cause synaptic dysfunction and subsequent loss [14,48,50,154,163,173].They are substantially smaller than plaques and can easily diffuse and spread [163]. It has been suggested that early memory loss in AD may be mediated by synaptic loss caused by oligomers [55]. Oligomers may cause cognitive symptoms by affecting long term potentiation and synaptic plasticity [28]. The putative oligomer Aβ*56 has been suggested to have a role in early AD [173]. It is associated with impaired memory in rats and mouse models of preclinical AD[173,174] which may happen independently of plaques and neuron loss[174] It is also elevated in individuals with an increased risk of AD and has been found to correlate with synapse dysfunction markers in cognitively normal individuals [173]. However, longitudinal studies are needed to determine its relation to cognitive decline [173]. Aggregates of tau oligomers have also been shown to correlate to memory loss in a mouse model of tauopathy [175]. Tau oligomers also correlate with cognitive decline in postmortem studies of MCI [48]. However, some antibodies against oligomeric forms of Aβ have failed in clinical trials[42].

5.5 EPISODIC MEMORY AND LANGUAGE IN EARLY AD

In our studies, we saw subtly worse performance primarily in episodic memory tests (Immediate and Delayed recall and Supra span, which can also measure attention) for participants with neurodegeneration or high levels of biomarkers for axonal / synaptic damage. Impairment in these tests is not surprising since memory is one of the first domains to be affected in early AD, especially episodic memory [176], which is related to recollection of experiences and events [177,178]. Episodic memory impairments have been shown already in the preclinical phase [176]. The recollection of experiences seem to be dependent on the medial temporal lobe [177,178] and the hippocampus[176] and its connection to the cortex, which are susceptible to aging-related and AD-related atrophy, possibly mediated by neuronal loss [156,178]. A considerable neuronal loss can be seen in these areas even in mild AD (individuals with CDR0.5). As much as 50% of the layer II entorhinal neurons can be lost.[178]

Apart from the hippocampus and the entorhinal cortex, neuropathological changes such as synapse loss in AD also occur in the frontal, temporal and parietal association cortices, which may have a relationship to semantic memory functions [179]. We also saw in the comparisons of individuals with A+ vs A- with CDR0.5 in study I that those with A+ scored slightly lower on the category fluency test than those with A-, but the groups scored similarly on letter fluency tests (Figure 7). Impairment in category fluency but less or no impairment in letter fluency seems to be common in AD [180-182] and can be seen early in the disease [183], but it is not clear why [179]. It has been suggested to depend on a degradation of semantic knowledge or semantic stores [180]. Although the category and letter fluency tests are similar, there are essential differences, which may explain why the category fluency test is more difficult for people with AD-related cognitive decline than the letter fluency test. It has been suggested that both tests depend on access to lexical / phonological stores and frontal retrieval processes [182], and according to a hypothesis, category fluency in addition relies on retrieval from other semantic stores that are distributed across the cortex and degrade during AD [182]. It has been hypothesized that people with AD perform worse on category fluency either due to the

disruption of these semantic stores, or the impairment of the mechanisms of retrieval from the semantic stores [182 180 184].

5.6 SUMMARY

In summary, the majority of our results showed that individuals with high vs low levels of biomarkers of AD, neurodegeneration and synapse loss were similar in cognitive tests. However, we also saw a slight cognitive decline in some individuals with high levels of biomarkers, especially biomarkers for neurodegeneration (tau, NfL) and synaptic damage (Ng). The results did not show a clear trend between cognitive test performance and amyloid (Study I and II) or amyloid and neurodegeneration levels (Study III). It is not clear what causes cognitive decline in AD, but one explanation could be synapse dysfunction or loss, possibly mediated by oligomers of amyloid and tau. The results from Study I and Study II suggest that some of the cognitive tests used in this thesis may be useful to demonstrate a slight cognitive decline in cognitively healthy older adults with AD biomarkers. However, since this study is more explorative than confirmatory, this needs to be further investigated, and should be seen as basis for future hypotheses.

5.7 METHODOLOGICAL CONSIDERATIONS

The results in this thesis are dependent on several factors relating to the methods used. We have used biomarker cutoffs developed by Hansson *et al*, that were determined by experts at Sahlgrenska University Hospital, that have been used before in our studies [142]. These cutpoints are validated against PET. However, abnormal values are difficult to define [33] and the lack of standards relating to biomarker cutoffs is a problem in the field of preclinical AD research, with variations across laboratories and countries [62,81]. Validating biomarkers and standardizing cutoffs is challenging, although there are ongoing international efforts [62,81].

The result can also be dependent on the assays used to measure the CSF biomarkers, as variations in assays may limit comparability between studies. However, the ELISA assays we have used to measure CSF $A\beta_{42}$, T-tau and P-tau are frequently used in the AD research field and as part of clinical routine, while the ELISAs for NfL and Ng are

in-house assays, developed at the Mölndal Clinical Neurochemistry Laboratory.
Another methodological issue is the binary division of biomarker variables into pathology vs non-pathology in the ATN system [76]. The pathology reflected by the biomarkers is a gradual process and unlike continuous variables, the binary division does not take this into account. Although a limitation, this binary division is common in diagnostic categorization [76]. However, individuals whose biomarker levels are close to the cutoff may be difficult to classify and interpret. The result might also depend on the cognitive test battery. The battery used in this thesis was developed for clinical psychological use in adults, [128], and it is not clear how suitable it is for measuring AD related cognitive decline in the general population. The Dureman-Sälde battery was developed during the 1950s, during a time in which the average cognitive capacity in the population may have been lower as the capacity has been suggested to rise with each generation (a phenomenon known as the Flynn effect [185]). However, the Flynn effect has been criticized for being unclear and containing methodological artifacts [186]. If the average cognitive capacity was slightly lower when the battery was developed, this could lead to ceiling effects in some tests. The MMSE especially has been criticized for having a ceiling effect, which is also visible in the slightly skew distribution of scores in the MMSE (Thurstone's picture memory test is also slightly skewed; however, the rest of the tests were normally distributed).
Another consideration regarding the cognitive tests is that their design could perhaps be optimized in future studies. One suggestion is that the design of the language tests may be improved by capturing more details of the test person's verbal ability, as this has been shown by a research group investigating animal fluency in relation to AD risk in cognitively normal participants from the African American Alzheimer's Disease Genetics Study [187]. The group found that when they used the word fluency animals test administered in the same way as in our battery (counting the total number of animals generated), the test did not predict genetic risk of AD (measured as having the *APOE ε4* allele) [187]. But the sensitivity of the test was increased when taking the complexity of the test persons vocabulary into account (the lexical frequency of the words generated) which then predicted AD risk successfully. This suggests that there could be additional linguistic information at the item-level of some cognitive tests, which could be

further explored in future research.
There may also be some overlap between the cognitive tests and domains, as some tests may belong in more than one cognitive domain, for example the test Word fluency could be seen as having executive components, and some of the memory tests also measure attention. The Digit span backward test that we have used to assess executive function can also be seen as a working memory /short term memory test [188].

5.8 STATISTICAL CONSIDERATIONS

We have during these studies performed many repetitions of t-tests which makes some type I errors (false positives) almost inevitable. The risk of this can be reduced by correction for multiple testing, however this instead increases the risk of type II errors (false negatives), especially if the correction method is conservative. P-values close to the significance level should therefore be interpreted with caution. Power analyses in Study I and II have shown that power was adequate to detect subtle differences between most of the groups, based on effect sizes of the level seen for the significant findings, but not based on effect sizes on the level seen for the non-significant results.
It is also difficult to know if the effect sizes observed are clinically relevant.

5.9 STRENGTHS AND LIMITATIONS

The strengths of these studies include the comprehensive examinations (performed by experienced research nurses trained by the PI) and the population-based design which is likely to make the study representative of the general population. This study design minimizes the risk of selection bias, although there could of course be differences between individuals who chose to take part in a study versus those who declined. Regarding this bias, some participants have reported poor health as a reason for declining participation, whereas others reported feeling too healthy as a reason for declining, which suggests that the sample is probably diverse in regards to participant health [2].
There could also be selection bias in the participation vs non-participation in LP, with healthier individuals more often included due to the fact that they have no contraindications. Those who were

excluded from LP could have a higher incidence of cancer or cerebrovascular risk factors.
Another limitation is that we did not have access to tau and amyloid PET.
All the studies in this thesis are cross-sectional, which of course limits the ability to draw conclusions. Follow-up studies will be needed in order to see if the participants develop cognitive decline. The results cannot be generalized to other populations such as younger age-groups or other nationalities, and may not be representative of the entire Swedish population since an inclusion criterion was the ability to speak Swedish.

5.10 CLINICAL IMPLICATIONS

5.10.1 THE UTILITY OF EARLY AD DIAGNOSIS

If disease-modifying treatments for AD are developed, they will need to be administered as early as possible, before neurodegeneration is too widespread [33]. It is not known whether prevention at this stage is effective [23] but it is an important area of research. One suggestion why so many therapeutic trials have failed could be the fact that most of the drugs have been tested in symptomatic individuals in which reversal or halting of the disease process might be too late [167]. It is therefore a desirable goal to develop new ways to diagnose AD as early as possible, including novel CSF, plasma, imaging and cognitive biomarkers. It is important to identify individuals who are suitable to take part in clinical trials for disease-modifying drugs, to give precedence to individuals with high AD risk who could benefit from treatment, and hence avoiding testing drugs that may have severe side effects on those who have a low probability of developing the disease. Interventions based on diet and other life-style related factors may also benefit individuals with an increased risk of developing AD [1], and it is also important to study if such lifestyle interventions work, and to accurately pinpoint people with a high likelihood of developing AD for inclusion in these studies [62]. If disease-modifying drugs become available, presymptomatic testing will be important to identify at risk individuals, and it is possible that cognitive tests could be used in combination with biomarkers or plasma assays, as this may enhance the diagnostic certainty or risk assessment.

5.11 CONTROVERSIES

5.11.1 PRESYMPTOMATIC TESTING

Presymptomatic testing is a controversial subject which is associated with a risk of adverse psychological effects when applied to incurable diseases, and the ethics and downsides of such testing has been discussed [33,189,190], Despite this, many people are positively inclined towards it and report that they are willing to undertake presymptomatic testing if available [22,191]. However, in the future, presymptomatic testing might become a necessity in order to enable early prevention of disease, similarly to other kinds of primary prevention such as healthy diet and physical activity for cardiovascular disease. Even though there is no treatment for AD, a survey showed that the majority of people responded that they wanted to undergo genetic testing to find out if they had a risk of developing the disease [191]. 90.5% also responded that they would pursue a healthier lifestyle if the test revealed that they had a high risk of AD, which illustrates the beneficial aspect of presymptomatic tests [191], as there seems to be a potential for modifying AD risk via life style changes[1]. However, 11.6% also said that they would seriously consider suicide [191]. The questionnaire also showed that many people failed to understand the implications of a positive genetic test, or did not accept the outcome of a positive test as evidence of the disease or increased risk of developing it [191]. Another study came to the conclusion that disclosing the *APOE* genotype to healthy individuals did not lead to any significant psychological risks [22,192]. Genetic tests are already available to consumers in some countries, but their clinical validity and utility is uncertain [191-193]. This highlights the need for discussing the ethical issue of presymtomatic testing, and educating the public about what positive results mean, which becomes increasingly important with advances in the preclinical AD field. Psychological screening has been suggested before administering genetic tests to interested individuals, as well as development of action plans to increase healthy lifestyle adaptations, as this is the primary rationale for participation in presymptomatic testing as long as there is no disease-modifying treatment [33,191]. Further research in this area is crucial so that the relationship between the CSF biomakers and cognition and/ or progression can be clearly elucidated.

6 CONCLUSION

This thesis found that older adults with T-tau pathology (N+) have increased axonal and synaptic damage and that older adults with preclinical AD had similar results in most cognitive tests as those without preclinical AD. Individuals with high levels of Nfl and Ng were also similar to those with lower levels. This could mean that there is little evidence for cognitive differences in people with and without AD/neurodegeneration biomarkers, or be a consequence of performing the study in a sample that was relatively young and healthy. However, some differences could be seen in some cognitive tests, primarily in memory tests. If it is possible to identify individuals on the AD pathway or with an increased risk of AD in the preclinical phase, it is important to investigate which cognitive tests may be suitable to use. In this thesis, we used the Dureman Sälde test battery, and found that some tests in the battery such as Delayed recall, Immediate recall, Supra span and MMSE may have potential to be used for detecting subtle changes in preclinical AD. However, this research is in an early phase towards the exploratory end, and the results need to be further confirmed in future studies.

7 FUTURE PERSPECTIVES

These studies have been cross-sectional, and findings need to be confirmed in future longitudinal studies. We need to do a follow up investigation to assess how many of the participants with preclinical AD will develop cognitive decline and progress to dementia. This would allow us to follow the possible trajectory of cognitive decline over time in the domains where we saw an impairment, for example by examining the participants with 5-year intervals.

ACKNOWLEDGEMENT

I want to express my sincerest gratitude to everyone who have been involved in this thesis and helped me throughout the years. There are many people without whom this work would not have been possible.

First and foremost, I want to thank **Ingmar Skoog** and **Silke Kern**, for giving me the opportunity to conduct my doctoral studies in this amazing group and for being my supervisors. I have learned so much from you, thank you for everything!

Silke, you have been an amazing main supervisor. Thank you for prioritizing my thesis and giving so much of your time. I really appreciate your positive and energetic attitude, your encouragement and inspiration, your help with editing my manuscripts and thesis, your feedback and insights, and your guidance throughout the project. I can't thank you enough for the time and effort you have put into this work. Thank you so much for your dedication and for helping me accomplishing my goal!

I also want to thank **Anna Zettergren**, my co-supervisor, for all that you have taught me throughout this project. Your feedback on my writing and your statistical knowledge have been invaluable.

Thank you **Kaj Blennow** and **Henrik Zetterberg** for giving valuable suggestions, feedback, and constructive criticism, and for sharing your experience and knowledge of the biomarker field.

I have been fortunate to have such incredible researchers as supervisors!

Thank you to all my knowledgeable and helpful **colleagues** in the EPINEP group who were always there for me to answer a myriad of questions. I appreciate that you introduced me to the field of epidemiology and showed me the ropes when I was a novice, and that you were always available whenever I needed help with scientific and practical matters – **Therese**, **Jenna**, **Hanna**, **Jessica**, **Felicia**, **Yadi**, **Mats**, **Madeleine**, **Lina**, **Mathias**, **Simona**, **Erik**, **Johan**, **Tobias**. I especially want to thank **Therese** and **Johan** for your feedback on my thesis, and my office mates **Isabell**, **Karolina** and **Simon**, for all our interesting discussions. Also, a special thanks to **Valter**, **Nazib**, **Yadi**

and **Tobias** for sharing your statistical expertise. I am very grateful for your advice, suggestions, and lessons.

I want to thank **all my colleagues** for the fun we've had during coffee breaks and over lunch – your company always brightens up the day and the congenial atmosphere here makes work so enjoyable.

I also want to express my gratitude to all my **co-authors** for your contributions to these papers. Your feedback and suggestions have been very appreciated. I have learned a lot from all of you and consider myself lucky to have such great co-workers.

Last but not least, I want to thank my **family**, and my **friends**, (you know who you are) especially **Daniel** for having such a keen interest in my thesis and such enthusiasm for science - thank you for being my sounding board and for all discussions and advice about research.

I also want to express my gratitude to the **study participants** – thank you for making this thesis possible!

REFERENCES

1 Livingston, G. *et al.* Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. *The Lancet* **396**, 413-446, doi:10.1016/S0140-6736(20)30367-6 (2020).

2 Rydberg Sterner, T. *et al.* The Gothenburg H70 Birth cohort study 2014-16: design, methods and study population. *European journal of epidemiology*, doi:10.1007/s10654-018-0459-8 (2018).

3 Blennow, K., de Leon, M. J. & Zetterberg, H. Alzheimer's disease. *Lancet (London, England)* **368**, 387-403, doi:10.1016/s0140-6736(06)69113-7 (2006).

4 Prince, M. World Alzheimer Report 2015. The Global Impact of Dementia. An Analysis of Prevalence, Incidence, Cost and Trends. (2015).

5 Lane, C. A., Hardy, J. & Schott, J. M. Alzheimer's disease. *European journal of neurology* **25**, 59-70, doi:10.1111/ene.13439 (2018).

6 Wilson, R. S. *et al.* Individual differences in rates of change in cognitive abilities of older persons. *Psychology and aging* **17**, 179-193 (2002).

7 Silverberg, N. B. *et al.* Assessment of cognition in early dementia. *Alzheimer's & dementia : the journal of the Alzheimer's Association* **7**, e60-e76, doi:10.1016/j.jalz.2011.05.001 (2011).

8 Joubert, S. *et al.* The cognitive and neural expression of semantic memory impairment in mild cognitive impairment and early Alzheimer's disease. *Neuropsychologia* **48**, 978-988, doi:10.1016/j.neuropsychologia.2009.11.019 (2010).

9 Bature, F., Guinn, B. A., Pang, D. & Pappas, Y. Signs and symptoms preceding the diagnosis of Alzheimer's disease: a systematic scoping review of literature from 1937 to 2016. *BMJ open* **7**, e015746, doi:10.1136/bmjopen-2016-015746 (2017).

10 Kolanko, M. A. & Malhotra, P. A. Exploring Alzheimer's disease subtypes at the prodromal stage. *Brain : a journal of neurology* **141**, 3285-3287, doi:10.1093/brain/awy282 (2018).

11 Wilson, R. S., Leurgans, S. E., Boyle, P. A. & Bennett, D. A. Cognitive decline in prodromal Alzheimer disease and mild cognitive impairment. *Archives of neurology* **68**, 351-356, doi:10.1001/archneurol.2011.31 (2011).

12 Grober, E. *et al.* Memory impairment, executive dysfunction, and intellectual decline in preclinical Alzheimer's disease. *Journal of the*

International Neuropsychological Society : JINS **14**, 266-278, doi:10.1017/s1355617708080302 (2008).

13 Johnson, D. K., Storandt, M., Morris, J. C. & Galvin, J. E. Longitudinal study of the transition from healthy aging to Alzheimer disease. *Archives of neurology* **66**, 1254-1259, doi:10.1001/archneurol.2009.158 (2009).

14 Ferreira, S. T. & Klein, W. L. The Aβ oligomer hypothesis for synapse failure and memory loss in Alzheimer's disease. *Neurobiology of Learning and Memory* **96**, 529-543, doi:https://doi.org/10.1016/j.nlm.2011.08.003 (2011).

15 Morris, J. C. *et al.* Mild cognitive impairment represents early-stage Alzheimer disease. *Archives of neurology* **58**, 397-405 (2001).

16 Petersen, R. C. *et al.* Mild cognitive impairment: a concept in evolution. *Journal of internal medicine* **275**, 214-228, doi:10.1111/joim.12190 (2014).

17 Petersen, R. C. Mild Cognitive Impairment. *Continuum (Minneap Minn)* **22**, 404-418, doi:10.1212/CON.0000000000000313 (2016).

18 Petersen, R. C. *et al.* Current concepts in mild cognitive impairment. *Archives of neurology* **58**, 1985-1992 (2001).

19 Morris, J. C. The Clinical Dementia Rating (CDR). *Current version and scoring rules* **43**, 2412-2412-a, doi:10.1212/WNL.43.11.2412-a (1993).

20 Perl, D. P. Neuropathology of Alzheimer's disease. *The Mount Sinai journal of medicine, New York* **77**, 32-42, doi:10.1002/msj.20157 (2010).

21 Spires-Jones, T. L., Kopeikina, K. J., Koffie, R. M., de Calignon, A. & Hyman, B. T. Are tangles as toxic as they look? *Journal of molecular neuroscience : MN* **45**, 438-444, doi:10.1007/s12031-011-9566-7 (2011).

22 Caselli, R. J., Beach, T. G., Knopman, D. S. & Graff-Radford, N. R. Alzheimer Disease: Scientific Breakthroughs and Translational Challenges. *Mayo Clinic proceedings* **92**, 978-994, doi:10.1016/j.mayocp.2017.02.011 (2017).

23 Sperling, R., Mormino, E. & Johnson, K. The Evolution of Preclinical Alzheimer's Disease: Implications for Prevention Trials. *Neuron* **84**, 608-622, doi:https://doi.org/10.1016/j.neuron.2014.10.038 (2014).

24 DeTure, M. A. & Dickson, D. W. The neuropathological diagnosis of Alzheimer's disease. *Molecular neurodegeneration* **14**, 32, doi:10.1186/s13024-019-0333-5 (2019).

25 Ow, S.-Y. & Dunstan, D. E. A brief overview of amyloids and Alzheimer's disease. *Protein Science* **23**, 1315-1331, doi:https://doi.org/10.1002/pro.2524 (2014).

26 Guo-fang, C. *et al.* Amyloid beta: structure, biology and structure-based therapeutic development. *Acta Pharmacologica Sinica* **38**, 1205–1235 (2017).
27 Jack, C. R., Jr. *et al.* Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *The Lancet. Neurology* **9**, 119-128, doi:10.1016/s1474-4422(09)70299-6 (2010).
28 Klein, W. L., Krafft, G. A. & Finch, C. E. Targeting small Abeta oligomers: the solution to an Alzheimer's disease conundrum? *Trends in neurosciences* **24**, 219-224, doi:10.1016/s0166-2236(00)01749-5 (2001).
29 Prvulovic, D. & Hampel, H. Amyloid β (Aβ) and phospho-tau (p-tau) as diagnostic biomarkers in Alzheimer's disease. *Clinical chemistry and laboratory medicine* **49**, 367-374, doi:10.1515/cclm.2011.087 (2011).
30 Niikura, T., Tajima, H. & Kita, Y. Neuronal cell death in Alzheimer's disease and a neuroprotective factor, humanin. *Current neuropharmacology* **4**, 139-147, doi:10.2174/157015906776359577 (2006).
31 Morris, M., Maeda, S., Vossel, K. & Mucke, L. The many faces of tau. *Neuron* **70**, 410-426, doi:10.1016/j.neuron.2011.04.009 (2011).
32 Schöll, M. *et al.* Biomarkers for tau pathology. *Molecular and cellular neurosciences* **97**, 18-33, doi:10.1016/j.mcn.2018.12.001 (2019).
33 Randall, C., Mosconi, L., de Leon, M. & Glodzik, L. Cerebrospinal fluid biomarkers of Alzheimer's disease in healthy elderly. *Front Biosci (Landmark Ed)* **18**, 1150-1173, doi:10.2741/4170 (2013).
34 Martin, L., Latypova, X. & Terro, F. Post-translational modifications of tau protein: implications for Alzheimer's disease. *Neurochemistry international* **58**, 458-471, doi:10.1016/j.neuint.2010.12.023 (2011).
35 Quinn, J. P., Corbett, N. J., Kellett, K. A. B. & Hooper, N. M. Tau Proteolysis in the Pathogenesis of Tauopathies: Neurotoxic Fragments and Novel Biomarkers. *Journal of Alzheimer's disease : JAD* **63**, 13-33, doi:10.3233/jad-170959 (2018).
36 Pooler, A. M., Noble, W. & Hanger, D. P. A role for tau at the synapse in Alzheimer's disease pathogenesis. *Neuropharmacology* **76 Pt A**, 1-8, doi:10.1016/j.neuropharm.2013.09.018 (2014).
37 Braak, H., Alafuzoff, I., Arzberger, T., Kretzschmar, H. & Del Tredici, K. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta neuropathologica* **112**, 389-404, doi:10.1007/s00401-006-0127-z (2006).
38 Serrano-Pozo, A., Frosch, M. P., Masliah, E. & Hyman, B. T. Neuropathological alterations in Alzheimer disease. *Cold Spring*

Harbor perspectives in medicine **1**, a006189, doi:10.1101/cshperspect.a006189 (2011).

39 Giannakopoulos, P. *et al.* Tangle and neuron numbers, but not amyloid load, predict cognitive status in Alzheimer's disease. *Neurology* **60**, 1495-1500, doi:10.1212/01.wnl.0000063311.58879.01 (2003).

40 Gao, Y. L. *et al.* Tau in neurodegenerative disease. *Annals of translational medicine* **6**, 175, doi:10.21037/atm.2018.04.23 (2018).

41 Skillbäck, T. *Biochemical markers in dementia - Exploring Swedish registry data and the human proteome*. (BrandFactory AB, 2019).

42 Liu, P.-P., Xie, Y., Meng, X.-Y. & Kang, J.-S. History and progress of hypotheses and clinical trials for Alzheimer's disease. *Signal Transduction and Targeted Therapy* **4**, 29, doi:10.1038/s41392-019-0063-8 (2019).

43 Du, X., Wang, X. & Geng, M. Alzheimer's disease hypothesis and related therapies. *Translational Neurodegeneration* **7**, 2, doi:10.1186/s40035-018-0107-y (2018).

44 Hardy, J. & Selkoe, D. J. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science (New York, N.Y.)* **297**, 353-356, doi:10.1126/science.1072994 (2002).

45 Hardy, J. A. & Higgins, G. A. Alzheimer's disease: the amyloid cascade hypothesis. *Science (New York, N.Y.)* **256**, 184-185, doi:10.1126/science.1566067 (1992).

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

47 Musiek, E. S. & Holtzman, D. M. Three dimensions of the amyloid hypothesis: time, space and 'wingmen'. *Nature neuroscience* **18**, 800-806, doi:10.1038/nn.4018 (2015).

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

49 Sadigh-Eteghad, S. *et al.* Amyloid-beta: a crucial factor in Alzheimer's disease. *Medical principles and practice : international journal of the Kuwait University, Health Science Centre* **24**, 1-10, doi:10.1159/000369101 (2015).

50 Mucke, L. & Selkoe, D. J. Neurotoxicity of amyloid β-protein: synaptic and network dysfunction. *Cold Spring Harbor perspectives in medicine* **2**, a006338-a006338, doi:10.1101/cshperspect.a006338 (2012).

51 Lee, H. G. *et al.* Challenging the amyloid cascade hypothesis: senile plaques and amyloid-beta as protective adaptations to Alzheimer

disease. *Annals of the New York Academy of Sciences* **1019**, 1-4, doi:10.1196/annals.1297.001 (2004).
52 Small, S. A. & Duff, K. Linking Abeta and tau in late-onset Alzheimer's disease: a dual pathway hypothesis. *Neuron* **60**, 534-542, doi:10.1016/j.neuron.2008.11.007 (2008).
53 Frost, B., Jacks, R. L. & Diamond, M. I. Propagation of tau misfolding from the outside to the inside of a cell. *The Journal of biological chemistry* **284**, 12845-12852, doi:10.1074/jbc.M808759200 (2009).
54 Mudher, A. *et al.* What is the evidence that tau pathology spreads through prion-like propagation? *Acta Neuropathologica Communications* **5**, 99, doi:10.1186/s40478-017-0488-7 (2017).
55 Lacor, P. N. *et al.* Synaptic targeting by Alzheimer's-related amyloid beta oligomers. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **24**, 10191-10200, doi:10.1523/jneurosci.3432-04.2004 (2004).
56 Shankar, G. M. *et al.* Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med* **14**, 837-842, doi:10.1038/nm1782 (2008).
57 Hansen, D. V., Hanson, J. E. & Sheng, M. Microglia in Alzheimer's disease. *The Journal of cell biology* **217**, 459-472, doi:10.1083/jcb.201709069 (2018).
58 Chew, H., Solomon, V. A. & Fonteh, A. N. Involvement of Lipids in Alzheimer's Disease Pathology and Potential Therapies. *Front Physiol* **11**, 598-598, doi:10.3389/fphys.2020.00598 (2020).
59 Shepardson, N. E., Shankar, G. M. & Selkoe, D. J. Cholesterol level and statin use in Alzheimer disease: I. Review of epidemiological and preclinical studies. *Archives of neurology* **68**, 1239-1244, doi:10.1001/archneurol.2011.203 (2011).
60 Mawanda, F. & Wallace, R. Can infections cause Alzheimer's disease? *Epidemiologic reviews* **35**, 161-180, doi:10.1093/epirev/mxs007 (2013).
61 Brucato, F. H. & Benjamin, D. E. Synaptic Pruning in Alzheimer's Disease: Role of the Complement System. *Glob J Med Res* **20**, 10.34257/gjmrfvol34220is34256pg34251, doi:10.34257/gjmrfvol20is6pg1 (2020).
62 Dubois, B. *et al.* Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. *Alzheimer's & dementia : the journal of the Alzheimer's Association* **12**, 292-323, doi:10.1016/j.jalz.2016.02.002 (2016).
63 McKhann, G. M. *et al.* The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for

Alzheimer's disease. *Alzheimer's & Dementia* **7**, 263-269, doi:https://doi.org/10.1016/j.jalz.2011.03.005 (2011).

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

65 Xu, W. *et al.* Meta-analysis of modifiable risk factors for Alzheimer's disease. *Journal of neurology, neurosurgery, and psychiatry* **86**, 1299-1306, doi:10.1136/jnnp-2015-310548 (2015).

66 Mayeux, R. & Stern, Y. Epidemiology of Alzheimer disease. *Cold Spring Harbor perspectives in medicine* **2**, doi:10.1101/cshperspect.a006239 (2012).

67 Stern, Y. Cognitive reserve in ageing and Alzheimer's disease. *The Lancet Neurology* **11**, 1006-1012, doi:https://doi.org/10.1016/S1474-4422(12)70191-6 (2012).

68 Roe, C. M. *et al.* Alzheimer disease and cognitive reserve: variation of education effect with carbon 11-labeled Pittsburgh Compound B uptake. *Archives of neurology* **65**, 1467-1471, doi:10.1001/archneur.65.11.1467 (2008).

69 van Loenhoud, A. C. *et al.* Cognitive reserve and clinical progression in Alzheimer disease. *A paradoxical relationship* **93**, e334-e346, doi:10.1212/wnl.0000000000007821 (2019).

70 Hörder, H. *et al.* Midlife cardiovascular fitness and dementia. *A 44-year longitudinal population study in women* **90**, e1298-e1305, doi:10.1212/wnl.0000000000005290 (2018).

71 Najar, J. *et al.* Cognitive and physical activity and dementia. *A 44-year longitudinal population study of women* **92**, e1322-e1330, doi:10.1212/wnl.0000000000007021 (2019).

72 Scarmeas, N., Levy, G., Tang, M. X., Manly, J. & Stern, Y. Influence of leisure activity on the incidence of Alzheimer's disease. *Neurology* **57**, 2236-2242, doi:10.1212/wnl.57.12.2236 (2001).

73 Bekris, L. M., Yu, C. E., Bird, T. D. & Tsuang, D. W. Genetics of Alzheimer disease. *Journal of geriatric psychiatry and neurology* **23**, 213-227, doi:10.1177/0891988710383571 (2010).

74 Yamazaki, Y., Zhao, N., Caulfield, T. R., Liu, C. C. & Bu, G. Apolipoprotein E and Alzheimer disease: pathobiology and targeting strategies. *Nature reviews. Neurology* **15**, 501-518, doi:10.1038/s41582-019-0228-7 (2019).

75 Sando, S. B. *et al.* APOE epsilon 4 lowers age at onset and is a high risk factor for Alzheimer's disease; a case control study from central Norway. *BMC neurology* **8**, 9-9, doi:10.1186/1471-2377-8-9 (2008).

76 Jack, C. R., Jr. *et al.* A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. *Neurology* **87**, 539-547, doi:10.1212/wnl.0000000000002923 (2016).
77 Jack, C. R., Jr. *et al.* NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* **14**, 535-562, doi:10.1016/j.jalz.2018.02.018 (2018).
78 Vos, S. J. *et al.* Preclinical Alzheimer's disease and its outcome: a longitudinal cohort study. *The Lancet. Neurology* **12**, 957-965, doi:10.1016/s1474-4422(13)70194-7 (2013).
79 Rajan, K. B., Wilson, R. S., Weuve, J., Barnes, L. L. & Evans, D. A. Cognitive impairment 18 years before clinical diagnosis of Alzheimer disease dementia. *Neurology* **85**, 898-904, doi:10.1212/wnl.0000000000001774 (2015).
80 Amieva, H. *et al.* The 9 year cognitive decline before dementia of the Alzheimer type: a prospective population-based study. *Brain : a journal of neurology* **128**, 1093-1101, doi:10.1093/brain/awh451 (2005).
81 Sperling, R. A. *et al.* Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* **7**, 280-292, doi:10.1016/j.jalz.2011.03.003 (2011).
82 Bateman, R. J. *et al.* Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *The New England journal of medicine* **367**, 795-804, doi:10.1056/NEJMoa1202753 (2012).
83 Aizenstein, H. J. *et al.* Frequent amyloid deposition without significant cognitive impairment among the elderly. *Archives of neurology* **65**, 1509-1517, doi:10.1001/archneur.65.11.1509 (2008).
84 Vemuri, P. *et al.* MRI and CSF biomarkers in normal, MCI, and AD subjects: predicting future clinical change. *Neurology* **73**, 294-301, doi:10.1212/WNL.0b013e3181af79fb (2009).
85 Jack, C. R., Jr. *et al.* 11C PiB and structural MRI provide complementary information in imaging of Alzheimer's disease and amnestic mild cognitive impairment. *Brain : a journal of neurology* **131**, 665-680, doi:10.1093/brain/awm336 (2008).
86 Delaère, P., He, Y., Fayet, G., Duyckaerts, C. & Hauw, J.-J. βA4 deposits are constant in the brain of the oldest old: An immunocytochemical study of 20 french centenarians. *Neurobiology of aging* **14**, 191-194, doi:https://doi.org/10.1016/0197-4580(93)90096-T (1993).

87 Dickson, D. W. *et al.* Identification of normal and pathological aging in prospectively studied nondemented elderly humans. *Neurobiology of aging* **13**, 179-189, doi:10.1016/0197-4580(92)90027-u (1992).
88 Jack, C. R. Preclinical Alzheimer's disease: a valid concept. *The Lancet Neurology* **19**, 31, doi:https://doi.org/10.1016/S1474-4422(19)30440-5 (2020).
89 Frisoni, G. B. *et al.* Re-aligning scientific and lay narratives of Alzheimer's disease. *The Lancet Neurology* **18**, 918-919, doi:https://doi.org/10.1016/S1474-4422(19)30323-0 (2019).
90 Dubois, B. *et al.* Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. *The Lancet. Neurology* **13**, 614-629, doi:10.1016/s1474-4422(14)70090-0 (2014).
91 Jack, C. R., Jr. *et al.* An operational approach to National Institute on Aging-Alzheimer's Association criteria for preclinical Alzheimer disease. *Annals of neurology* **71**, 765-775, doi:10.1002/ana.22628 (2012).
92 Olsson, B. *et al.* CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *The Lancet. Neurology* **15**, 673-684, doi:10.1016/s1474-4422(16)00070-3 (2016).
93 Blennow, K. & Zetterberg, H. Biomarkers for Alzheimer's disease: current status and prospects for the future. *Journal of internal medicine* **284**, 643-663, doi:10.1111/joim.12816 (2018).
94 Janelidze, S. *et al.* Cerebrospinal fluid p-tau217 performs better than p-tau181 as a biomarker of Alzheimer's disease. *Nature Communications* **11**, 1683, doi:10.1038/s41467-020-15436-0 (2020).
95 Buerger, K. *et al.* CSF tau protein phosphorylated at threonine 231 correlates with cognitive decline in MCI subjects. *Neurology* **59**, 627-629, doi:10.1212/wnl.59.4.627 (2002).
96 Blennow, K. *et al.* Tau protein in cerebrospinal fluid: a biochemical marker for axonal degeneration in Alzheimer disease? *Molecular and chemical neuropathology* **26**, 231-245, doi:10.1007/bf02815140 (1995).
97 Sjögren, M. *et al.* Both total and phosphorylated tau are increased in Alzheimer's disease. *Journal of neurology, neurosurgery, and psychiatry* **70**, 624-630, doi:10.1136/jnnp.70.5.624 (2001).
98 Buerger, K. *et al.* CSF phosphorylated tau protein correlates with neocortical neurofibrillary pathology in Alzheimer's disease. *Brain : a journal of neurology* **129**, 3035-3041, doi:10.1093/brain/awl269 (2006).
99 Hampel, H. *et al.* Total and phosphorylated tau protein as biological markers of Alzheimer's disease. *Experimental gerontology* **45**, 30-40, doi:10.1016/j.exger.2009.10.010 (2010).

100 Schoonenboom, N. S. *et al.* Cerebrospinal fluid markers for differential dementia diagnosis in a large memory clinic cohort. *Neurology* **78**, 47-54, doi:10.1212/WNL.0b013e31823ed0f0 (2012).
101 Hesse, C. *et al.* Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. *Neuroscience letters* **297**, 187-190, doi:10.1016/s0304-3940(00)01697-9 (2001).
102 Otto, M. *et al.* Tau protein and 14-3-3 protein in the differential diagnosis of Creutzfeldt-Jakob disease. *Neurology* **58**, 192-197, doi:10.1212/wnl.58.2.192 (2002).
103 Zetterberg, H. *et al.* Neurochemical aftermath of amateur boxing. *Archives of neurology* **63**, 1277-1280, doi:10.1001/archneur.63.9.1277 (2006).
104 Cicognola, C. *et al.* Novel tau fragments in cerebrospinal fluid: relation to tangle pathology and cognitive decline in Alzheimer's disease. *Acta neuropathologica* **137**, 279-296, doi:10.1007/s00401-018-1948-2 (2019).
105 Khalil, M. *et al.* Neurofilaments as biomarkers in neurological disorders. *Nature reviews. Neurology* **14**, 577-589, doi:10.1038/s41582-018-0058-z (2018).
106 Gaetani, L. *et al.* Neurofilament light chain as a biomarker in neurological disorders. *Journal of neurology, neurosurgery, and psychiatry* **90**, 870-881, doi:10.1136/jnnp-2018-320106 (2019).
107 Mielke, M. M. *et al.* Plasma and CSF neurofilament light: Relation to longitudinal neuroimaging and cognitive measures. *Neurology* **93**, e252-e260, doi:10.1212/wnl.0000000000007767 (2019).
108 Zetterberg, H. *et al.* Association of Cerebrospinal Fluid Neurofilament Light Concentration With Alzheimer Disease Progression. *JAMA neurology* **73**, 60-67, doi:10.1001/jamaneurol.2015.3037 (2016).
109 Hoffman, P. N. *et al.* Neurofilament gene expression: a major determinant of axonal caliber. *Proceedings of the National Academy of Sciences of the United States of America* **84**, 3472-3476 (1987).
110 Skillback, T. *et al.* CSF neurofilament light differs in neurodegenerative diseases and predicts severity and survival. *Neurology* **83**, 1945-1953, doi:10.1212/wnl.0000000000001015 (2014).
111 Norgren, N., Rosengren, L. & Stigbrand, T. Elevated neurofilament levels in neurological diseases. *Brain research* **987**, 25-31 (2003).
112 Teunissen, C. E. & Khalil, M. Neurofilaments as biomarkers in multiple sclerosis. *Multiple sclerosis (Houndmills, Basingstoke, England)* **18**, 552-556, doi:10.1177/1352458512443092 (2012).
113 Pereira, J. B., Westman, E. & Hansson, O. Association between cerebrospinal fluid and plasma neurodegeneration biomarkers with

brain atrophy in Alzheimer's disease. *Neurobiology of aging* **58**, 14-29, doi:10.1016/j.neurobiolaging.2017.06.002 (2017).

114 Kern, S. *et al.* Association of Cerebrospinal Fluid Neurofilament Light Protein With Risk of Mild Cognitive Impairment Among Individuals Without Cognitive Impairment. *JAMA neurology*, doi:10.1001/jamaneurol.2018.3459 (2018).

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

116 Petersen, A. & Gerges, N. Z. Neurogranin regulates CaM dynamics at dendritic spines. *Scientific reports* **5**, 11135, doi:10.1038/srep11135 (2015).

117 Portelius, E. *et al.* Cerebrospinal fluid neurogranin: relation to cognition and neurodegeneration in Alzheimer's disease. *Brain : a journal of neurology* **138**, 3373-3385, doi:10.1093/brain/awv267 (2015).

118 Portelius, E. *et al.* Cerebrospinal fluid neurogranin concentration in neurodegeneration: relation to clinical phenotypes and neuropathology. *Acta neuropathologica* **136**, 363-376, doi:10.1007/s00401-018-1851-x (2018).

119 Camporesi, E. *et al.* Fluid Biomarkers for Synaptic Dysfunction and Loss. *Biomarker insights* **15**, 1177271920950319, doi:10.1177/1177271920950319 (2020).

120 Tarawneh, R. *et al.* Diagnostic and Prognostic Utility of the Synaptic Marker Neurogranin in Alzheimer Disease. *JAMA neurology* **73**, 561-571, doi:10.1001/jamaneurol.2016.0086 (2016).

121 Kester, M. I. *et al.* Neurogranin as a Cerebrospinal Fluid Biomarker for Synaptic Loss in Symptomatic Alzheimer Disease. *JAMA neurology* **72**, 1275-1280, doi:10.1001/jamaneurol.2015.1867 (2015).

122 Becker, B. *et al.* Alzheimer-associated cerebrospinal fluid fragments of neurogranin are generated by Calpain-1 and prolyl endopeptidase. *Molecular neurodegeneration* **13**, 47, doi:10.1186/s13024-018-0279-z (2018).

123 Wellington, H. *et al.* Increased CSF neurogranin concentration is specific to Alzheimer disease. *Neurology* **86**, 829-835, doi:10.1212/wnl.0000000000002423 (2016).

124 Kern, S. *et al.* Prevalence of preclinical Alzheimer disease: Comparison of current classification systems. *Neurology* **90**, e1682-e1691, doi:10.1212/wnl.0000000000005476 (2018).

125 AmericanPsychiatricAssociation. Diagnostic and statistical manual of mental disorders, 5th ed. *Washington DC American Psychiatric Press* (2013).

126 AmericanPsychiatricAssociation. Diagnostic and statistical manual of mental disorders,3rd ed *American Psychiatric Press* (1987).
127 AmericanPsychiatricAssociation. Diagnostic and statistical manual of mental disorders (DSM-III-R). (1987).
128 Österberg, I. D. L. K. E. Manual till DS-batteriet (Manual for the DS-battery). *Psykologiförlaget AB* (1971).
129 Thorvaldsson, V., Karlsson, P., Skoog, J., Skoog, I. & Johansson, B. Better Cognition in New Birth Cohorts of 70 Year Olds, But Greater Decline Thereafter. *The journals of gerontology. Series B, Psychological sciences and social sciences* **72**, 16-24, doi:10.1093/geronb/gbw125 (2017).
130 Buschke, H. & Fuld, P. A. Evaluating storage, retention, and retrieval in disordered memory and learning. *Neurology* **24**, 1019-1025, doi:10.1212/wnl.24.11.1019 (1974).
131 Thurstone, L. L., Thurstone TG. Manual to SRA Primary Mental Abilities. *Chicago: Science Research Associates.* (1949).
132 Shao, Z., Janse, E., Visser, K. & Meyer, A. S. What do verbal fluency tasks measure? Predictors of verbal fluency performance in older adults. *Frontiers in Psychology* **5**, doi:10.3389/fpsyg.2014.00772 (2014).
133 Folstein, M. F., Folstein, S. E. & McHugh, P. R. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *Journal of psychiatric research* **12**, 189-198, doi:10.1016/0022-3956(75)90026-6 (1975).
134 Berg, L. Clinical Dementia Rating (CDR). *Psychopharmacology bulletin* **24**, 637-639 (1988).
135 Hughes, C. P., Berg, L., Danziger, W. L., Coben, L. A. & Martin, R. L. A new clinical scale for the staging of dementia. *The British journal of psychiatry : the journal of mental science* **140**, 566-572 (1982).
136 O'Bryant, S. E. *et al.* Staging dementia using Clinical Dementia Rating Scale Sum of Boxes scores: a Texas Alzheimer's research consortium study. *Archives of neurology* **65**, 1091-1095, doi:10.1001/archneur.65.8.1091 (2008).
137 Vanmechelen, E. *et al.* Quantification of tau phosphorylated at threonine 181 in human cerebrospinal fluid: a sandwich ELISA with a synthetic phosphopeptide for standardization. *Neuroscience letters* **285**, 49-52 (2000).
138 Andreasen, N. *et al.* Cerebrospinal fluid beta-amyloid(1-42) in Alzheimer disease: differences between early- and late-onset Alzheimer disease and stability during the course of disease. *Archives of neurology* **56**, 673-680 (1999).

139 Palmqvist, S. *et al.* Accuracy of brain amyloid detection in clinical practice using cerebrospinal fluid β-amyloid 42: a cross-validation study against amyloid positron emission tomography. *JAMA neurology* **71**, 1282-1289, doi:10.1001/jamaneurol.2014.1358 (2014).

140 Gaetani, L. *et al.* A new enzyme-linked immunosorbent assay for neurofilament light in cerebrospinal fluid: analytical validation and clinical evaluation. *Alzheimer's research & therapy* **10**, 8, doi:10.1186/s13195-018-0339-1 (2018).

141 Hoglund, K. *et al.* Preclinical amyloid pathology biomarker positivity: effects on tau pathology and neurodegeneration. *Translational psychiatry* **7**, e995, doi:10.1038/tp.2016.252 (2017).

142 Hansson, O. *et al.* Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. *The Lancet. Neurology* **5**, 228-234, doi:10.1016/s1474-4422(06)70355-6 (2006).

143 Roberts, R. O. *et al.* Prevalence and Outcomes of Amyloid Positivity Among Persons Without Dementia in a Longitudinal, Population-Based Setting. *JAMA neurology* **75**, 970-979, doi:10.1001/jamaneurol.2018.0629 (2018).

144 Petersen, R. C. *et al.* Association of Elevated Amyloid Levels With Cognition and Biomarkers in Cognitively Normal People From the Community. *JAMA neurology* **73**, 85-92, doi:10.1001/jamaneurol.2015.3098 (2016).

145 Knopman, D. S. *et al.* Short-term clinical outcomes for stages of NIA-AA preclinical Alzheimer disease. *Neurology* **78**, 1576-1582, doi:10.1212/WNL.0b013e3182563bbe (2012).

146 Vos, S. J. B. *et al.* NIA-AA staging of preclinical Alzheimer disease: discordance and concordance of CSF and imaging biomarkers. *Neurobiology of aging* **44**, 1-8, doi:10.1016/j.neurobiolaging.2016.03.025 (2016).

147 Skoog, I. *et al.* Cerebrospinal fluid beta-amyloid 42 is reduced before the onset of sporadic dementia: a population-based study in 85-year-olds. *Dementia and geriatric cognitive disorders* **15**, 169-176, doi:10.1159/000068478 (2003).

148 Jack, C. R., Jr. *et al.* Suspected non-Alzheimer disease pathophysiology--concept and controversy. *Nature reviews. Neurology* **12**, 117-124, doi:10.1038/nrneurol.2015.251 (2016).

149 Casaletto, K. B. *et al.* Neurogranin, a synaptic protein, is associated with memory independent of Alzheimer biomarkers. *Neurology* **89**, 1782-1788, doi:10.1212/wnl.0000000000004569 (2017).

150 Bliss, T. V. P. & Collingridge, G. L. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361**, 31-39, doi:10.1038/361031a0 (1993).

151 Jackson, J. *et al.* Targeting the Synapse in Alzheimer's Disease. *Frontiers in Neuroscience* **13**, doi:10.3389/fnins.2019.00735 (2019).
152 Scheff, S. W., Price, D. A., Schmitt, F. A. & Mufson, E. J. Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. *Neurobiology of aging* **27**, 1372-1384, doi:10.1016/j.neurobiolaging.2005.09.012 (2006).
153 John, A. & Reddy, P. H. Synaptic basis of Alzheimer's disease: Focus on synaptic amyloid beta, P-tau and mitochondria. *Ageing Research Reviews* **65**, 101208, doi:https://doi.org/10.1016/j.arr.2020.101208 (2021).
154 de Wilde, M. C., Overk, C. R., Sijben, J. W. & Masliah, E. Meta-analysis of synaptic pathology in Alzheimer's disease reveals selective molecular vesicular machinery vulnerability. *Alzheimer's & dementia : the journal of the Alzheimer's Association* **12**, 633-644, doi:10.1016/j.jalz.2015.12.005 (2016).
155 Jadhav, S. *et al.* Tau-mediated synaptic damage in Alzheimer's disease. *Translational neuroscience* **6**, 214-226, doi:10.1515/tnsci-2015-0023 (2015).
156 Morrison, J. H. & Baxter, M. G. The ageing cortical synapse: hallmarks and implications for cognitive decline. *Nature reviews. Neuroscience* **13**, 240-250, doi:10.1038/nrn3200 (2012).
157 Blennow, K., Bogdanovi , N., Alafuzoff, I., Ekman, R. & Davidsson, P. Synaptic pathology in Alzheimer's disease: Relation to severity of dementia, but not to senile plaques, neurofibrillary tangles, or the ApoE4 allele. *Journal of Neural Transmission* **103**, 603-618 (2005).
158 Terry, R. D. *et al.* Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Annals of neurology* **30**, 572-580, doi:10.1002/ana.410300410 (1991).
159 DeKosky, S. T. & Scheff, S. W. Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. *Annals of neurology* **27**, 457-464, doi:10.1002/ana.410270502 (1990).
160 Hayashi, Y. Long-term potentiation: two pathways meet at neurogranin. *The EMBO Journal* **28**, 2859-2860, doi:https://doi.org/10.1038/emboj.2009.273 (2009).
161 Mukhin, V. N., Pavlov, K. I. & Klimenko, V. M. Mechanisms of Neuron Loss in Alzheimer's Disease. *Neuroscience and Behavioral Physiology* **47**, 508-516, doi:10.1007/s11055-017-0427-x (2017).
162 Terry, R. D. Cell Death or Synaptic Loss in Alzheimer Disease. *Journal of Neuropathology & Experimental Neurology* **59**, 1118-1119, doi:10.1093/jnen/59.12.1118 (2000).

163 Verma, M., Vats, A. & Taneja, V. Toxic species in amyloid disorders: Oligomers or mature fibrils. *Ann Indian Acad Neurol* **18**, 138-145, doi:10.4103/0972-2327.144284 (2015).

164 Stéphan, A., Laroche, S. & Davis, S. Generation of aggregated beta-amyloid in the rat hippocampus impairs synaptic transmission and plasticity and causes memory deficits. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **21**, 5703-5714, doi:10.1523/jneurosci.21-15-05703.2001 (2001).

165 Perez-Nievas, B. G. *et al.* Dissecting phenotypic traits linked to human resilience to Alzheimer's pathology. *Brain : a journal of neurology* **136**, 2510-2526, doi:10.1093/brain/awt171 (2013).

166 Hyman, B. T. Amyloid-dependent and amyloid-independent stages of Alzheimer disease. *Archives of neurology* **68**, 1062-1064, doi:10.1001/archneurol.2011.70 (2011).

167 Mehta, D., Jackson, R., Paul, G., Shi, J. & Sabbagh, M. Why do trials for Alzheimer's disease drugs keep failing? A discontinued drug perspective for 2010-2015. *Expert opinion on investigational drugs* **26**, 735-739, doi:10.1080/13543784.2017.1323868 (2017).

168 Bancher, C., Lassmann, H., Breitschopf, H. & Jellinger, K. A. Mechanisms of cell death in Alzheimer's disease. *Journal of neural transmission. Supplementum* **50**, 141-152, doi:10.1007/978-3-7091-6842-4_14 (1997).

169 Masliah, E., Hansen, L., Albright, T., Mallory, M. & Terry, R. D. Immunoelectron microscopic study of synaptic pathology in Alzheimer's disease. *Acta neuropathologica* **81**, 428-433, doi:10.1007/bf00293464 (1991).

170 Honer, W. G., Dickson, D. W., Gleeson, J. & Davies, P. Regional synaptic pathology in Alzheimer's disease. *Neurobiology of aging* **13**, 375-382, doi:10.1016/0197-4580(92)90111-a (1992).

171 Masliah, E., Mallory, M., Hansen, L., DeTeresa, R. & Terry, R. D. Quantitative synaptic alterations in the human neocortex during normal aging. *Neurology* **43**, 192-197, doi:10.1212/wnl.43.1_part_1.192 (1993).

172 Lambert, M. P. *et al.* Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 6448-6453, doi:10.1073/pnas.95.11.6448 (1998).

173 Zahs, K. & Ashe, K. β-Amyloid oligomers in aging and Alzheimer's disease. *Frontiers in Aging Neuroscience* **5**, doi:10.3389/fnagi.2013.00028 (2013).

174 Lesné, S. *et al.* A specific amyloid-β protein assembly in the brain impairs memory. *Nature* **440**, 352-357, doi:10.1038/nature04533 (2006).
175 Berger, Z. *et al.* Accumulation of pathological tau species and memory loss in a conditional model of tauopathy. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **27**, 3650-3662, doi:10.1523/jneurosci.0587-07.2007 (2007).
176 Bäckman, L., Small, B. J. & Fratiglioni, L. Stability of the preclinical episodic memory deficit in Alzheimer's disease. *Brain : a journal of neurology* **124**, 96-102, doi:10.1093/brain/124.1.96 (2001).
177 Dickerson, B. C. & Eichenbaum, H. The Episodic Memory System: Neurocircuitry and Disorders. *Neuropsychopharmacology* **35**, 86-104, doi:10.1038/npp.2009.126 (2010).
178 Gallagher, M. & Koh, M. T. Episodic memory on the path to Alzheimer's disease. *Curr Opin Neurobiol* **21**, 929-934, doi:10.1016/j.conb.2011.10.021 (2011).
179 Salmon, D. P., Butters, N. & Chan, A. S. The deterioration of semantic memory in Alzheimer's disease. *Canadian journal of experimental psychology = Revue canadienne de psychologie experimentale* **53**, 108-117, doi:10.1037/h0087303 (1999).
180 Henry, J. D., Crawford, J. R. & Phillips, L. H. Verbal fluency performance in dementia of the Alzheimer's type: a meta-analysis. *Neuropsychologia* **42**, 1212-1222, doi:10.1016/j.neuropsychologia.2004.02.001 (2004).
181 Salmon, D. P., Heindel, W. C. & Lange, K. L. Differential decline in word generation from phonemic and semantic categories during the course of Alzheimer's disease: implications for the integrity of semantic memory. *Journal of the International Neuropsychological Society : JINS* **5**, 692-703 (1999).
182 Cerhan, J. H. *et al.* Diagnostic utility of letter fluency, category fluency, and fluency difference scores in Alzheimer's disease. *The Clinical neuropsychologist* **16**, 35-42, doi:10.1076/clin.16.1.35.8326 (2002).
183 Cottingham, M. E. & Hawkins, K. A. Verbal fluency deficits co-occur with memory deficits in geriatric patients at risk for dementia: Implications for the concept of mild cognitive impairment. *Behavioural neurology* **22**, 73-79, doi:10.3233/ben-2009-0246 (2010).
184 Butters, N., Granholm, E., Salmon, D. P., Grant, I. & Wolfe, J. Episodic and semantic memory: a comparison of amnesic and demented patients. *Journal of clinical and experimental neuropsychology* **9**, 479-497, doi:10.1080/01688638708410764 (1987).

185 Williams, R. L. Overview of the Flynn effect. *Intelligence* **41**, 753-764, doi:https://doi.org/10.1016/j.intell.2013.04.010 (2013).
186 Rodgers, J. L. A critique of the Flynn Effect: massive IQ gains, methodological artifacts, or both? *Intelligence* **26**, 337-356, doi:https://doi.org/10.1016/S0160-2896(99)00004-5 (1998).
187 Vonk, J. M. J. *et al.* Semantic network function captured by word frequency in nondemented APOE ε4 carriers. *Neuropsychology* **33**, 256-262, doi:10.1037/neu0000508 (2019).
188 Clair-Thompson, H. S. Backwards digit recall: A measure of short-term memory or working memory? *European Journal of Cognitive Psychology* (2009).
189 McConnell, L. M., Koenig, B. A., Greely, H. T. & Raffin, T. A. Genetic testing and Alzheimer disease: Has the time come? *Nature Medicine* **4**, 757-759, doi:10.1038/nm0798-757 (1998).
190 National Institute on Aging/Alzheimer's Association Working, G. & Relkin, N. Apolipoprotein E genotyping in Alzheimer's disease. *The Lancet* **347**, 1091-1095, doi:https://doi.org/10.1016/S0140-6736(96)90284-6 (1996).
191 Caselli, R. J. *et al.* Public perceptions of presymptomatic testing for Alzheimer disease. *Mayo Clinic proceedings* **89**, 1389-1396, doi:10.1016/j.mayocp.2014.05.016 (2014).
192 Green, R. C. *et al.* Disclosure of APOE Genotype for Risk of Alzheimer's Disease. *New England Journal of Medicine* **361**, 245-254, doi:10.1056/NEJMoa0809578 (2009).
193 McGuire, A. L. & Burke, W. An unwelcome side effect of direct-to-consumer personal genome testing: raiding the medical commons. *Jama* **300**, 2669-2671, doi:10.1001/jama.2008.803 (2008).