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Acknowledgements

Three fast-paced years have passed since my friend, Ragna Espenes, asked if I would be interested in taking up a vacant position as a PhD candidate in the DDI project where she worked. Today I am extremely happy that I said yes to that opportunity. This thesis has really been a labor of love, and I often find myself working on research ideas, doing statistical analyses or writing papers in weekends and even sometimes when I am supposed to be on holidays! Not because I have to, because I want to and love to do the work.

None of this enthusiasm and love of work would exist without the excellent support of my supervisors these past three years. First and foremost Professor Knut Waterloo, who has always been available to help and support with whatever I have needed, and literally at all hours! Professor Tormod Fladby at Ahus/UiO and leader of the national DDI project has been a key supporter in everything I have done, and especially in mentoring me on the biological foundations of Alzheimer’s disease and encouraging me to pursue new research ideas. Professor Erik Hessen has had a central role throughout, lending his extensive expertise in the field to further my understanding of brain-behavior relationships through his impressive knowledge of neuropsychological research in Alzheimer’s disease.

I am also very thankful for our fantastic DDI team in Tromsø who shares in the enthusiasm of the research and making DDI Tromsø run and work smoothly! Stein Harald Johnsen, Grit Richter, Ragna Espenes, Johan Jacob Espenes, Ingrid Myrvoll Lorentzen and Torgil Vangberg. Also I want to thank our clinical supporters at the Department of Neurology Marianne Røst, Grethe Berg Johnsen, Elisabeth Gundersen, Claus Albretsen, Kai Ivar Muller and Kjell Arne Arntzen for help with clinical examinations and Tom Sollid at the UNN laboratory for essential support, and of importance, Svein Bekkelund, who initially started and led the DDI initiative in Tromsø.

DDI is a national cooperation, and this thesis was supported by research groups in
Trondheim, Stavanger and Oslo/Akershus. I particularly thank Per Selnes, Kaja Nordengen, Jonas Alexander Jarholm, Lene Pålhaugen, Silje Bøen Torsetnes, Berglind Gísladóttir, Marianne Wettergren, Erna Utnes, Sandra Tecelao, Santiago Timón, Sigrid Botne Sando, Gøril Rolfseng Grøntvedt, Geir Bråthen and Dag Aarsland for their continued help, support and collaboration.

To my family and friends, thank you for your kind words of encouragement and support throughout this journey! I especially thank my loving girlfriend Alise Marie for her fantastic support throughout my PhD journey and the many hours she has had to listen to my probably “fascinating” monologues on APP dysmetabolism, synapse loss, microglia, linear regression models and memory loss. While my PhD journey is at an end, I still hope you will manage to endure future “interesting” monologues in the years to come from yours truly.
**List of papers**


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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>Aβ</td>
<td>Beta-Amyloid</td>
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<tr>
<td>Aβ+</td>
<td>Pathological amyloid beta 42 in the cerebrospinal fluid</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>AβPP</td>
<td>Amyloid Precursor Protein</td>
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<tr>
<td>BACE1</td>
<td>β-site APP-cleaving enzyme 1</td>
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<tr>
<td>CERAD</td>
<td>Consortium to Establish a Registry for Alzheimer’s Disease</td>
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<tr>
<td>COWAT</td>
<td>Controlled Oral Word Association Test</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal Fluid</td>
</tr>
<tr>
<td>DDI</td>
<td>Dementia Disease Initiation</td>
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<tr>
<td>MCI</td>
<td>Mild Cognitive Impairment</td>
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<tr>
<td>MMSE</td>
<td>Mini Mental State Examination</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MTL</td>
<td>Medial Temporal Lobe</td>
</tr>
<tr>
<td>NIA-AA</td>
<td>National Institute on Aging–Alzheimer’s Association</td>
</tr>
<tr>
<td>Ng</td>
<td>Neurogranin</td>
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<tr>
<td>Ng/BACE1</td>
<td>Neurogranin/BACE1 Ratio</td>
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<tr>
<td>PET</td>
<td>Positron emission tomography</td>
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<tr>
<td>P-Tau</td>
<td>Phosphorylated Tau</td>
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<td>SCD</td>
<td>Subjective Cognitive Decline</td>
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<tr>
<td>TMT</td>
<td>Trail Making Test</td>
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<td>T-tau</td>
<td>Total Tau</td>
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<td>VOSP</td>
<td>Visual Object and Space Perception</td>
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Abstract

**Background:** Alzheimer’s disease (AD) may develop 10-15 years before onset of mild cognitive impairment (MCI). Early intervention may serve to halt or delay disease progression. Thus, there is a need to investigate early cognitive and biological markers to detect and track disease progression. Subjective cognitive decline (SCD) is an established risk-factor for AD. However, SCD is a common phenomenon in healthy aging, and most cases are benign. Thus, improved methods of identifying and tracking SCD due to AD are needed.

**Objectives/aims:** This thesis investigates the role of SCD as a preclinical stage of AD and seeks to improve methods of early detection. In paper I, potential recruitment source biases in demographics and cognitive performance between memory-clinic referred and self-referred SCD and MCI cases were investigated. In paper II, the cerebrospinal fluid (CSF) Neurogranin/BACE1 ratio was explored as a biomarker of putatively AD-coupled synapse affection in SCD and MCI cases with amyloid plaques. In paper III, more sensitive and culturally adapted test norms for the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) word list episodic memory test (WLT) was developed.

**Methods:** Participants were primarily drawn from the Norwegian “Dementia Disease Initiation (DDI)” study comprising 658 baseline and 428 follow-up participants. An additional 59 healthy controls were included from the Norwegian “Trønderbrain” study for the purpose of developing cognitive test norms.

**Results and conclusions:** In paper I, we found that both the SCD and MCI groups, regardless of recruitment method, showed reduced cognitive performance compared to controls. Differences in cognitive impairment for memory clinic-referrals compared to self-referrals were found only within the MCI group. In this study, a need to establish new test norms for the episodic memory test, CERAD WLT was revealed, which were ultimately developed in...
paper III. The CSF Neurogranin/BACE1 ratio was increased in SCD and MCI cases with amyloid plaques. Increased ratios were related to reductions in hippocampal and amygdala volumes, corresponding to cognitive impairment at baseline and decline at 2-year follow-up. The Neurogranin/BACE1 ratio holds promise as a preclinical AD marker of synapse loss.

1 Introduction

More than a century has passed since Alois Alzheimer first described “A peculiar severe disease process of the cerebral cortex”. Where upon autopsy, the brain histology of a 50-year-old woman showed distinct plaques and neurofibrillary tangles (Hippius & Neundörfer, 2003). Plaques and tangles were later identified as consisting of beta-amyloid proteins and abnormally folded tau proteins (Kosik, Joachim, & Selkoe, 1986; Masters et al., 1985). In the early 1990’s, the amyloid cascade hypothesis was first described (D. J. Selkoe, 1991). While other views exist (Kametani & Hasegawa, 2018; Small & Duff, 2008), the amyloid hypothesis is to date the dominant model of AD pathogenesis. This hypothesis states that the accumulation of beta-amyloid (Aβ) due to reduced or failure of Aβ clearance mechanisms sets of a detrimental cascade of events, ultimately leading to the formation of neurofibrillary tangles, loss of synapses and neuronal degradation which cause cognitive impairment and dementia (Dennis J. Selkoe & Hardy, 2016). In addition, several lines of evidence implicate the innate immune system as a potential key player in the AD pathological trajectory (Fan, Brooks, Okello, & Edison, 2017; Jansen et al., 2019; Nordengen et al., 2019; Rajendran & Paolicelli, 2018).

Alzheimer’s Disease (AD) has been extensively studied, especially the past four decades, with many discoveries being made, but unfortunately so far not resulting in effective treatments. AD is by far the most common cause of dementia, accounting for between 50-75 % of cases
Dementia and cognitive impairment are the leading chronic disease contributors to disability and care dependency among older people worldwide (Livingston et al., 2017). Dementia is primarily an age-related condition, and as populations are ageing in most countries, the frequency of dementia is increasing and prevalence rates are expected to double every 20 years (Prince et al., 2013). The cost to patients, caregivers and society as a whole is immense. Global costs was estimated at 604 billion USD in 2010 (Wimo, Jonsson, Bond, Prince, & Winblad, 2013), and a recent Swedish report estimates a societal cost of 0.5 million NOK yearly for each patient with dementia (Akerborg et al., 2016). In 2014, The Norwegian public health report estimated dementia prevalence to 80 000 – 100 000 ("Public Health Report: Dementia in Norway," 2014) which would equate to costs of approximately 40 – 50 billion NOK annually. With numbers expected to increase, it is therefore of paramount importance to discover methods, which may prevent, stabilize or reduce prevalence rates. The discovery of effective prevention or intervention measures will be of huge benefit for patients, caregiver and society as a whole.

1.1 The biological continuum of Alzheimer’s Disease

Alzheimer’s disease (AD) may be described as a biological continuum that includes the hallmark pathological processes of amyloid-beta (Aβ) dysmetabolism, formation of amyloid plaques (A), neurofibrillary tangles (T) and neurodegeneration (N), which may be derived from measuring cerebrospinal fluid (CSF) levels of Aβ1-42, phosphorylated tau (p-tau) and total-tau (t-tau), respectively (C. R. Jack, et al., 2018). While most regard amyloid dysmetabolism and plaque formation as an early event in the AD disease trajectory, the precise pathophysiological mechanisms and sequence of events from early formation of amyloid plaque towards the formation of neurofibrillary tangles, synapse degeneration and neuronal loss are not yet fully understood (C. R. Jack et al., 2018; Marsh & Alifragis, 2018).
To aid research efforts in delineating the evolution of AD pathology, C. R. Jack et al. (2018) have proposed an unbiased classification system for AD biomarkers, which summarize the presence or absence of pathological markers as an A/T/N-score. This score can be used to classify cases along the AD biological continuum according to severity of pathological change. For example, the sole presence of amyloid plaque pathology would yield a A+T-N-score, whereas the presence of pathological neurodegeneration and neurofibrillary tangle formation would yield a A+T+N+ score (C. R. Jack et al., 2018).

Previous research has largely focused on the pathological changes linked to cognitive impairment, either in the early stages of mild cognitive impairment (MCI), or at the later stage of dementia. However, converging evidence from studies of at-risk cohorts and clinically normal older individuals indicates that the pathophysiological underpinnings of Alzheimer’s disease may begin 10 to 15 years before the emergence of clinical symptoms (Perrin, Fagan, & Holtzman, 2009). Consequently, this has led to the proposal that AD has a preclinical phase wherein brain-compensatory mechanisms make up for early pathological changes (Dubois et al., 2016; Sperling, Aisen, et al., 2011). Intervention studies aimed at reducing parenchymal amyloid plaque load has generally shown no improvement in cognition (Honig et al., 2018; Ostrowitzki et al., 2012; Salloway et al., 2014). A contributing factor to this lack of success may be due to the inclusion of patients late in the trajectory of the disease, where substantial and possibly irreversible loss of neurons and cognitive dysfunction have already occurred. Future effective treatments in the preclinical phase of the disease (i.e. before clinical cognitive impairment) could serve to preserve cognitive function or delay onset of cognitive decline (Karran & De Strooper, 2016; Reiman et al., 2016; Sperling, Aisen, et al., 2011). Thus, identifying individuals at risk for AD in the preclinical phase is a key objective (Dubois et al., 2016; Jessen et al., 2014; Sperling, Jack, & Aisen, 2011).
1.2 Clinical manifestation of preclinical AD: Subjective Cognitive Decline

A proposed target population for preclinical AD is patients with subjective experience of cognitive deficits, hypothesizing that subjective cognitive decline (SCD), while performing within the normal range on standardized cognitive tests, may imply risk of having abnormal AD CSF biomarkers and show greater progression towards MCI and ultimately AD dementia (Jessen et al., 2014). SCD should manifest before the onset of MCI or dementia, and could potentially serve as a target population for early intervention trials. Indeed, several longitudinal studies have shown that SCD carries a small, but detectable risk of conversion to MCI (Mendonca, Alves, & Bugalho, 2016; Ronnlund, Sundstrom, Adolfsson, & Nilsson, 2015; van Harten et al., 2013; Visser et al., 2009). However, an overwhelming majority do not show progression to objective cognitive decline (MCI or Dementia) when assessed at follow-up (Hessen et al., 2017; Mendonca et al., 2016). Indeed, it has been shown that 43% of those aged between 65 and 74 years report subjective memory problems, while dementia prevalence in this age range is low (Bassett & Folstein, 1993). Thus, in many, if not most cases, the experience of cognitive decline may be benign. Several studies have shown that the presence of biomarkers indicating amyloid plaque deposition in cognitively normal individuals carries an increased risk of progression to MCI (Petersen et al., 2016; van Harten et al., 2013; Vogel et al., 2017). However, identification of pathological biomarkers presently requires invasive and costly procedures through biomarker CSF analysis or amyloid PET imaging. Consequently, there is a need to identify the characteristics of SCD due to AD and other disorders to identify preclinical at-risk populations eligible for early intervention and intervention trials (Jessen et al., 2014).

The Subjective Cognitive Decline working group (SCD-I) (Jessen et al., 2014) has proposed a conceptual framework for research on SCD as a preclinical risk factor for AD. Among several
issues, they underline that differences in research setting, design and participant selection may influence the composition of clinical characteristics within at-risk cohorts. At-risk participants are recruited by different means, resulting in cohorts with different clinical and demographic characteristics. It has been demonstrated that MCI patients recruited through memory clinics are cognitively more impaired (Brodaty et al., 2014), show a higher prevalence of *APOE* ε4 alleles (Brodaty et al., 2014; Fladby et al., 2017), harbor more AD-type pathology (Fladby et al., 2017; Whitwell et al., 2012), and show higher risk of progression to dementia (Farias, Mungas, Reed, Harvey, & DeCarli, 2009; Roh et al., 2016) compared to study participants recruited through community or population based samples. However, few studies have investigated the effects of recruitment bias for patients with SCD (Rodriguez-Gomez, Abdelnour, Jessen, Valero, & Boada, 2015). Chen et al. (2016) demonstrated that persons with normal cognitive scores at baseline, showed an annual conversion rate to MCI of 30% in a memory clinic sample compared to 5% in a community-based sample. The authors attributed this finding to level of concern leading to medical help seeking. Similarly, Perrotin et al. (2016) found reduced cerebral gray matter volumes and increased depressive symptomatology in SCD cases from a memory clinic sample compared to a community sample. While these studies did not demonstrate any differences in cognitive performance due to recruitment bias in SCD cases, Abdelnour et al. (2017) showed reduced cognitive performance in SCD cases from a memory-unit compared to cases recruited from an open house initiative offering free examinations to the community. These findings demonstrate a need to explore potential differences in clinical characteristics within and between preclinical cohorts employing different recruitment strategies. SCD is a particularly vulnerable clinical group, as many cases ultimately are not related to AD (Bassett & Folstein, 1993; Hessen et al., 2017; Mendonca et al., 2016).
1.3 The measurement of cognitive deficits due to AD

In order to determine clinical stage (e.g. cognitively normal SCD or impaired MCI/Dementia) and measure clinical progression in AD, standardized tests of cognitive performance within several cognitive domains are employed (e.g. memory, attention and executive functions, language and visuoperceptual abilities). MCI in elderly persons has been studied extensively the past decades (Petersen, 2016). MCI is conceived as a prodromal phase of AD and other neurodegenerative disorders, where patients show mild deficits on standardized tests of cognitive performance while still retaining the ability to function independently in their daily lives (Albert et al., 2011). Memory impairment is the most prominent feature of prodromal AD, with most cases either showing mild impairments in episodic memory (pure amnestic MCI) or memory impairment with concurrent deficits in other cognitive domains such as attention and executive functions (amnestic multidomain MCI) (Petersen, 2016). The latter is often associated with increased neurodegenerative burden (Lenzi et al., 2011; Whitwell et al., 2007), and more rapid progression to dementia (Hessen et al., 2014; Nordlund et al., 2010; Tabert et al., 2006). However, the time of disease onset and clinical progression varies considerably due to differences in genetic and environmental risk factors (Gatz et al., 2006; Jansen et al., 2019; Reitz & Mayeux, 2014; Tosto et al., 2017). Furthermore, some cases of MCI may be caused by conditions other than neurodegenerative disease (Petersen, 2016). Moreover, it has been shown that people with higher levels of education, or with a history of intellectually challenging work, may be more resistant against AD pathological change. This is known as the “cognitive reserve hypothesis”, whereby some individuals may better adjust to the effects of synapse loss and neuronal degradation in the earlier phases of the disease and thus retain normal performance on cognitive tests (Stern, 2012). Alternatively, individuals with high cognitive reserve may have a higher premorbid baseline due to superior cognitive function, and while declining from their individual baseline levels, still perform within the
accepted normal range on cognitive tests at clinical assessment (Soldan et al., 2017). Indeed, there is support for a “threshold effect” where individuals with higher education may resist the detrimental effects of neurodegeneration for a longer period of time, but show more rapid progression in cognitive decline once brain pathology reaches a critical level (Meng & D'Arcy, 2012). In addition, while advancing age is associated with decline in episodic memory performance (Park & Festini, 2017), tests of verbal list learning memory such as the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) wordlist test (WLT) also show a female advantage in normative performance (Beeri et al., 2006; Heaton, Miller, Taylor, & Grant, 2004; Liu et al., 2011). If left unchecked, these factors could influence estimates of cognitive performance and consequently incorrectly diagnose individuals as cognitively impaired, or cognitively normal. More importantly, MCI due to AD may remain undetected, and are thus precluded from entry in intervention trials.

In order to reliably measure normative performance of cognitive functions, clinicians rely on published norms, which aim to correct for demographics known to influence test performance. The CERAD WLT is a widely used word list memory test in AD research. However, it was originally developed to detect AD dementia, and MCI due to AD in at-risk geriatric populations. Thus, norms are primarily developed for elderly cohorts (Beeri et al., 2006; Fillenbaum et al., 2005; Sotaniemi et al., 2012; Welsh et al., 1994). More recent research efforts now focus on tracking the preclinical or asymptomatic phases of the AD trajectory. Consequently, several slightly younger cohorts have been established (Fladby et al., 2017; Soldan et al.; Weiner et al., 2015). Recently, Hankee et al. (2016) proposed norms for the CERAD WLT for younger and middle-aged adults based on an American sample. These norms are aimed at younger individuals (<55 years), and norms are only provided for either age or education. However, as learning and memory are influenced by age, education,
and gender (Beeri et al., 2006; Heaton et al., 2004; Liu et al., 2011) correction for additional demographic factors may be necessary to avoid misclassification of cognitively normal and impaired individuals. In addition, CERAD WLT norms developed for Scandinavian countries (Danish, Swedish or Norwegian language) are lacking. Thus, in order to reliably detect MCI and track cognitive decline in younger cohorts, more sensitive and culturally adapted norms for cognitive tests, including the CERAD WLT, may need to be established.

A conventional approach to establish norms for cognitive tests is the use of discrete norming procedures (e.g. capturing the normative performance of a certain demographic as a reference group). However, to ensure that the reference group is a representative sample of the population distribution, this approach requires an adequate sample size of healthy individuals. When adjusting for several demographics such as age, gender and education, the sample size requirements increase dramatically (Oosterhuis, van der Ark, & Sijtsma, 2016). In addition, normative performance may increase or decrease substantially by moving from one reference age group to the next (i.e. moving from a 54-59 year group to 60-65 year group) (Zachary & Gorsuch, 1985). A possible solution is to use a regression-based continuous norming procedure (Parmenter, Testa, Schretlen, Weinstock-Guttman, & Benedict, 2010; Testa, Winicki, Pearlson, Gordon, & Schretlen, 2009). Using multiple regression analyses, this approach uses the entire normative reference sample to estimate the relative effects of demographics such as age, gender and educational influences on CERAD WLT performance. As a consequence of using the entire normative sample to estimate demographic influences, the sample size requirements are 2.5 to 5.5 times smaller than by conventional discrete norming procedures (Oosterhuis et al., 2016). The derived regression equations from this analysis may be used to estimate predicted normative performance. More importantly, this approach allows highly individualized norms due to the adjustment of several covariates in a
linear fashion, meaning that the estimation of normative performance is possible at yearly increases in age and education for both males and females. Using this approach, the individual differences in performance should largely be due to factors other than known demographic influences, such as subtle or mild cognitive deficits due to pathology in the preclinical and prodromal stages of AD.

1.4 Synapse loss in Alzheimer’s disease, an early event?

While increased levels of CSF t-tau have been established as a marker of neuronal loss (C. R. Jack et al., 2018), several lines of research indicate loss of synaptic integrity and function as an early event in AD (Alberdi et al., 2010; Alzheimer's Association Calcium Hypothesis, 2017; Dennis J. Selkoe, 2002; Zhang, Li, Feng, & Wu, 2016). Thus, sensitive markers of synaptic affection due to AD are sought. Moreover, synaptic function is closely related to cognition (Terry et al., 1991), and early synaptic affection may relate to the cognitive deficits seen in early mild cognitive impairment (MCI) even before substantial neuronal loss has occurred (Lleo et al., 2019).

Neurogranin is a post-synaptic protein, which is highly expressed in dendritic spines of hippocampal and amygdala pyramidal cells and is linked to post-synaptic signal transduction and long-term potentiation of memories through the dendritic spine NMDA Ca\(^{2+}\)-Calmodulin second messenger complex (Diez-Guerra, 2010; Higo, Oishi, Yamashita, Matsuda, & Hayashi, 2004). Increased levels of CSF neurogranin (Ng) have been related to loss of synapses and elevated levels of CSF Ng have been found in both MCI and dementia with amyloid plaques compared to both healthy controls and other neurodegenerative diseases (Kester et al., 2015; Portelius et al., 2015; Tarawneh et al., 2016; Wellington et al., 2016).

While synaptic loss is not specific to AD, the apparent specificity of neurogranin related
synapse loss in AD may be due to its prominent expression in the pyramidal cells of medial temporal lobe (MTL) structures such as the hippocampus (Higo et al., 2004) and thus relate to the observed memory deficits in AD. In AD, amyloid-β precursor protein (AβPP) metabolizes to Aβ-peptide, which precipitate in amyloid plaques (Vassar, 2004). In a recent study, an inverse relationship between CSF Ng and the CSF Aβ1-42/Aβ1-40 ratio in MCI and dementia was shown, suggesting that synaptic loss and AβPP metabolism may be linked (De Vos et al., 2015). The β-site APP cleaving enzyme 1 (BACE1) is a rate-limiting step in the production of beta amyloid through its metabolism of AβPP and is largely found in presynaptic terminals (Del Prete, Lombino, Liu, & D'Adamio, 2014; Sun & Roy, 2018). A known genetic risk factor for AD is the presence of one (heterozygote) or two (homozygote) APOE ε4 alleles, which is linked to AD through several proposed pathways. An important AD related pathway is through its interaction with the β-amyloid precursor protein (AβPP) which has shown to both increase availability of AβPP (Huang, Zhou, Wernig, & Sudhof, 2017) and increase the propensity of soluble monomers of Aβ1-42 to form oligomers (Huynh, Davis, Ulrich, & Holtzman, 2017; Sanan et al., 1994). In experimental studies, Aβ-oligomers have been shown to accumulate at synaptic terminals where it disrupts pyramidal cell N-methyl-D-aspartate (NMDA) receptors leading to post-synaptic Ca^{2+} dyshomeostasis, (Alberdi et al., 2010; Alzheimer's Association Calcium Hypothesis, 2017; Zhang et al., 2016) which putatively lead to loss of synapses.

In a recent study, several CSF measures were compared as both single analytes and ratios to cognitive decline. It was demonstrated that an increased ratio between CSF neurogranin trunc P75 and BACE1 (CSF Ng/BACE1) was a robust correlate of cognitive decline in MCI cases due to AD (e.g. with amyloid plaques) (De Vos et al., 2016). Since BACE1 is predominately a presynaptic enzyme, and neurogranin is located in post-synaptic spines, these proteins are
highly correlated. De Vos et al. (2016) argued that this ratio may reflect synaptic integrity and thus relate to cognition. However, this ratio may alternatively reflect an Aβ-linked disease mechanism whereby the release of post-synaptic neurogranin in CSF (reflecting synapse loss), is related to the toxic effect of Aβ oligomers at the synaptic terminals. As the pre-synaptic activity of BACE1 relates to rate of Aβ production, the relative increase in CSF Ng/BACE1 ratio may be a sensitive candidate marker of early synapse affection in AD. Increased levels of this ratio could herald development of cognitive deficits even at a preclinical stage of AD.

2 Objectives

The overall objective of this thesis was to investigate the role of SCD as a preclinical stage of AD and to improve methods of early detection of at-risk individuals. Herein, I aimed to investigate methods to improve the identification of at-risk SCD cases that are due to AD, develop more sensitive norms for the detection and tracking of normative episodic memory performance and investigate a new CSF biomarker of putatively AD-coupled synapse affection that may closely relate to both subjective and objective cognitive decline or impairment. Paper I investigates potential recruitment biases in cognitive performance and demographics in SCD and MCI participants recruited through memory-clinic referred participants as compared to self-referred participants following response to advertisements in media, newspapers or news bulletins. Paper II investigates if the CSF Ng/BACE1 ratio is increased in SCD and MCI cases with amyloid plaques and relate to reduced magnetic resonance imaging (MRI) derived MTL volumetry, cognitive deficits and longitudinal decline, putatively due to synaptotoxic Aβ oligomers. Paper III seeks to develop demographically adjusted CERAD WLT test norms in a Norwegian sample aged 40 – 80
years using a regression-based norming procedure.

3 Methods and materials

3.1 The Dementia Disease Initiation Cohort

Participants were primarily drawn from the national multi-center study “Dementia Disease Initiation” (DDI) cohort comprising inclusions from university hospitals in the Norwegian health regions (Helse Sør-Øst, Helse Vest, Helse Midt and Helse Nord). Between January 2013 and February 2019, participants with self-reported cognitive reduction and healthy controls were recruited. In early 2017, when drafting paper I, the cohort comprised a total of 577 participants of which n=463 fulfilled inclusion criteria and had completed assessments. As the DDI study is still including participants, the cohort is growing. In 2018, when papers II and III were drafted, the cohort grew to n=744 subjects (n=658 fulfilling inclusion criteria with completed assessments), and n=428 had available 2-year follow-up assessments with 4 year follow-ups just starting. Participant inclusion according to papers I-III is illustrated in Figure 1. Participants were recruited mainly from general practitioner (GP) referrals to local memory clinics, or self-referred following advertisements in media, newspapers or news bulletins. Healthy controls were recruited from spouses of patients with cognitive symptoms, volunteers from the community responding to advertisements, newspapers or news bulletins, and from patients who completed lumbar puncture for orthopedic surgery. All participants were examined following a standardized comprehensive assessment protocol and staged as either healthy controls, SCD or MCI using published criteria (Albert et al., 2011; Jessen et al., 2014) (described below). Individuals with a native language of Norwegian, Swedish or Danish were included. In order to capture individuals in the preclinical, as well as prodromal phases of AD, participants between 40 and 80 years of age were included. Exclusion criteria were brain trauma or disorder, including clinical stroke, dementia, severe psychiatric disorder,
severe somatic disease that might influence the cognitive functions, or intellectual disability or other developmental disorders.

3.2 The Trønderbrain Cohort

For the purposes of paper III, an additional 59 healthy controls were included from the Trønderbrain cohort. This cohort recruited participants with MCI, early AD dementia and healthy controls between 2009 and 2015. Healthy controls were recruited from societies for retired people in central Norway, or spouses of recruited MCI or early AD dementia participants. Exclusion criteria were a present psychiatric or malignant disease (i.e. currently undergoing treatment for cancer), use of anticoagulant medication or high alcohol consumption (Berge et al., 2016).

3.3 DDI Case report form and cognitive screening battery

The DDI case report form (CRF) includes a comprehensive account of the participants' medical history (corroborated by an informant when possible) as well as physical and neurological examinations and a measure of depressive symptoms using the 15-item Geriatric Depression Scale (GDS) (Mitchell, Bird, Rizzo, & Meader, 2010). Educational level was encoded in two ways. 1) Recorded as a continuous measure of total years of education and 2) Classified according to norms provided by Heaton et al. (2004) in the following categories: 0 = Primary school (7 – 8 years), 1 = High School (9 – 11 years), 2 = College (12 years), 3 = Bachelor degree (13-15 years), 4 = Master or equivalent = 16 – 17 years, 5 = Higher university degree/PhD (18 - 20 years). The cognitive assessment battery included the Mini Mental State Examination (MMSE-NR) (Folstein, Folstein, & McHugh, 1975), non-verbal cognitive screening (The clock drawing test) (Shulman, 2000), verbal learning & memory
(CERAD WLT) (Fillenbaum et al., 2008), visuoperceptual ability (VOSP silhouettes) (Warrington & James, 1991), psychomotor speed and attention/executive functions (Trail making test (TMT) A and B) and the Controlled Oral Word-Association Test (COWAT), a measure of word fluency (Benton & Hamsher, 1989).

3.4 Classification of healthy controls, SCD and MCI

The CRF includes an account of participants’ experience of subjective cognitive decline modeled on the suggested framework by the working group of SCD-I. It includes the nature of cognitive decline (cognitive domain, onset), concerns and worries including feeling worse compared to age matched peers and informant confirmation of decline (when available).

Participants were classified as SCD according to the SCD-I framework, which requires normal objective cognitive performance in combination with subjectively experienced decline in any cognitive domain (Jessen et al., 2014). MCI was classified according to the NIA-AA criteria, which require the presence of subjective cognitive decline combined with cognitive impairment in one or more cognitive domains, yet preserved independence in functional ability and not fulfilling the criteria of dementia (Albert et al., 2011; McKhann et al., 2011).

Healthy controls did not endorse any subjective experience of cognitive decline. Performance was classified as normal or abnormal according to published norms for the different tests (Benton & Hamsher, 1989; Fillenbaum et al., 2008; Folstein et al., 1975; Reitan & Wolfson, 1985; Shulman, 2000; Sotaniemi et al., 2012; Warrington & James, 1991). Due to overlapping and mutually exclusive criteria, the cut-off values for SCD vs. MCI (defined as normal or abnormal cognition) were ≤1.5 standard deviation below normative mean on either CERAD WLT (delayed recall), VOSP silhouettes, TMT-B or COWAT, or having MMSE score equal to or below 27. Cognitive functioning was also assessed by the Clinical Dementia
Rating scale (CDR) (Hughes, Berg, Danziger, Coben, & Martin, 1982). Participants with dementia were excluded if CDR > 0.5 (Petersen, 2004).

3.5 Cerebrospinal fluid (CSF) and blood biomarkers

The standard assessment protocol includes collection of CSF and blood biomarkers from controls, SCD and MCI cases. However, biomarkers were only analyzed in paper II. CSF biomarkers were collected through lumbar puncture (performed before noon), using polypropylene tubes (Thermo Nunc) and centrifuged within 4h at 2000g for 10min at room temperature. The supernatant was transferred to new tubes and frozen at −80°C prior to analysis. All CSF samples were analyzed at the Department of Interdisciplinary Laboratory Medicine and Medical Biochemistry at Akershus University Hospital, and samples from other sites were frozen before sending to this laboratory.

CSF Aβ1-42, total tau, and phosphorylated tau were determined using ELISA (Innotest β-Amyloid (1–42), Innotest h-Tau Ag and Innotest Phospho-Tau (181P), Fujirebio, Ghent, Belgium). CSF BACE1 and neurogranin (trunc P75) levels were determined using kits from EUROIMMUN AG (Lübeck, Germany) and are described in detail elsewhere (De Vos et al., 2016). All samples were analyzed in duplicates and reanalyzed if relative deviations (RDs) exceeded 20% and quality control samples with RD threshold of 15% controlled for interplate and interday variation.

APOE genotyping was performed on EDTA blood samples either at Akershus University Hospital (Gene Technology Division, Department of Interdisciplinary Laboratory Medicine and Medical Biochemistry) according to the laboratory’s routine protocol using real-time
PCR combined with a TaqMan assay (Applied Biosystems, Thermo Fisher Scientific, Waltham, USA) or at the University Hospital of Trondheim according to the protocol for the Fast Start DNA Master HybProbe Kit (Roche, Basel, Switzerland) in combination with the LightMix ApoE C112R R158C kit from TiB MolBiol (Berlin, Germany) followed by LightCycler technology (Roche, Basel, Switzerland).

3.6 A/T/N classification

In paper II, participants were classified according to the A/T/N classification scheme for AD using CSF biomarkers (C. R. Jack et al., 2018). Where A+ denote (CSF amyloid pathology only), A+N+ (CSF amyloid pathology and neurodegenerative marker) and A+T+N+ (CSF amyloid pathology, neurodegenerative marker and marker of neurofibrillary tangles). An optimal cut-off at CSF Aβ<sub>1-42</sub> <708 for amyloid plaque pathology was determined following DDI PET [<sup>18</sup>F]-Flutemetamol uptake studies (Kalheim, Fladby, Coello, Bjørnerud, & Selnos, 2018). The following cut-off values for CSF total tau (t-tau) and phosphorylated tau (p-tau) abnormality were applied according to the laboratory recommendations (modified from Sjogren et al. (2001)); t-tau >300 pg/ml for age <50 years, >450 pg/ml for age 50–69 years, and >500 pg/ml for age ≥70 years and p-tau ≥80 pg/ml.

3.7 Magnetic resonance imaging (MRI)

All participants in DDI were referred to MRI scan. However, in this thesis, brain MRI images were only acquired and analyzed in paper II. MRI was performed at seven sites, and seven scanners were used, a total of 57 MRI scans out of 74 included cases were available for analysis. For group 1 (12 subjects), MRI was performed on a Philips Achieva 3 Tesla system (Philips Medical Systems, Best, The Netherlands). A 3D T1-weighted turbo field echo
sequence (TR/TE/TI/FA = 4.5 ms/2.2 ms/853 ms/8° matrix = 256 × 213, 170 slices, thickness = 1.2 mm, in-plane resolution of 1 mm × 1.2 mm) was obtained. For group 2 (22 subjects), MRI was performed on a Philips Ingenia 3 Tesla system (Philips Medical Systems, Best, The Netherlands). A 3D T1-weighted turbo field echo sequence (TR/TE/TI/FA = 4.5 ms/2.2 ms/853 ms/8°, matrix = 256 × 213, 170 slices, thickness = 1.2 mm, in-plane resolution of 1 mm × 1.2 mm) was obtained. For group 3 (3 subjects), MRI was performed on a Siemens Skyra 3 Tesla system (Siemens Medical Solutions, Erlangen, Germany). A 3D T1-Magnetization-Prepared Rapid Gradient-Echo sequence (TR/TE/TI/FA = 2300 ms/2.98 ms/900 ms/9° matrix = 256 × 256, 176 slices, thickness = 1.2 mm, in-plane resolution of 1.0 mm × 1.0 mm) was obtained. For group 4 (11 subjects), MRI was performed on a Philips Ingenia 1.5 Tesla system (Philips Medical Systems, Best, The Netherlands). A 3D T1-weighted turbo field echo sequence (TR/TE/TI/FA = 7.63 ms/3.49 ms/937 ms/8° matrix = 256 × 256, 180 slices, thickness = 1.0 mm, in-plane resolution of 1.0 mm × 1.0 mm) was obtained. For group 5 (1 subject), MRI was performed on a Siemens Avanto 1.5 Tesla system (Siemens Medical Solutions, Erlangen, Germany). A 3D T1-weighted Magnetization-Prepared Rapid Gradient-Echo sequence (TR/TE/TI/FA = 1190 ms/3.10 ms/750 ms/15° matrix = 512 × 512, 144 slices, thickness = 1.0 mm, in-plane resolution of 0.50 mm × 0.50 mm) was obtained. For group 6 (7 subjects), MRI was performed on a GE Optima Medical Systems 1.5 Tesla system (GE Healthcare, Chicago, Illinois, USA). A 3D T1-weighted fast spoiled gradient echo sequence (TR/TE/TI/FA = 11.26 ms/5.04 ms/500 ms/10° matrix = 256 × 256, 156 slices, thickness = 1.2 mm, in-plane resolution of 1.0 mm × 1.0 mm). Lastly, one MRI scan was performed on a Siemens Avanto 1.5 Tesla system (Siemens Medical Solutions, Erlangen, Germany). A 3D T1-weighted Magnetization-Prepared Rapid Gradient-Echo sequence (TR/TE/TI/FA = 1700 ms/2.42 ms/1000 ms/15° matrix = 256 × 256, 144 slices, thickness = 1.2 mm, in-plane resolution of 1.0 mm × 1.0 mm) was obtained.
3.8 MRI segmentations and analyses

Volumetric segmentation was performed with the FreeSurfer image analysis suite version 6.0.0 (http://surfer.nmr.mgh.harvard.edu/). This includes segmentation of the subcortical white matter and deep gray matter volumetric structures (Fischl et al., 2002). For the hippocampus and amygdala, volumes from the left and right hemispheres were added, and relative volumes (per ml of total intracranial volume) were computed.

3.9 Ethics

The regional medical research ethics committee approved the study. Participants gave their written informed consent before taking part in the study. All further study conduct was in line with the guidelines provided by the Helsinki declaration of 1964, revised 2013 and the Norwegian Health and Research Act.

3.10 Participant selection according to papers I-III

Participant selections according to papers I-III are illustrated in Figure 1.

For Paper I, a total of n=577 participants with baseline data were considered, and 87 were excluded due to withdrawal or not fulfilling the baseline criteria. Of the remaining 490 participants, 463 had disease stage classification available. Of these, 32 controls had abnormal cognitive screening and were excluded from analysis. This yielded a total of 431 subjects comprising healthy controls (n= 132), SCD (n=163) and MCI, n=136). A total of n=179 cases were self-referred (recruited through response to advertisements), and n=86 were recruited from local memory clinics. Participants recruited by other means were excluded from analysis.
(n=34). For **paper II**, a total of n=74 participants were selected from the DDI cohort according to study design criteria: 1) Healthy controls with low risk of AD (n = 20, APOE-ε4-), 2) Healthy controls with increased risk of AD (at least one APOE-ε4 allele and first degree relative with dementia, n = 16, APOE-ε4+), 3) SCD (n = 18) with CSF confirmed amyloid pathology, 4) MCI (n = 20) with CSF confirmed amyloid pathology. In addition, n=42 had come to 2 year follow-up examinations. Amyloid-positive cases were screened in accordance with the A/T/N classification scheme (C. R. Jack et al., 2018) before inclusion to ensure equal distribution of pathological markers between SCD and MCI groups. For **paper III**, a total of n=227 healthy controls were included from the DDI cohort (n=168) and the “Trønderbrain” cohort (n=59). In addition, n= 168 participants with MCI from the DDI cohort was included.

Figure 1. Participant selections from the DDI and Trønderbrain cohort according to papers I-III
3.11 Statistical analyses

All statistical analyses for papers I-III were performed with the Statistical Package for the Social Sciences (SPSS version 24 and 25). For both paper 1 & II, normality was assessed through the visual inspection of QQ-plots, box-plots, histograms of frequency distributions and the Shapiro-Wilk test of normality. Effect sizes were reported for ANOVAs ($\eta^2_p$) Mann-Whitney U tests and Kruskal-Wallis tests ($\eta^2$) (Fritz, Morris, & Richler, 2012).

3.11.1 Paper I.

For continuous variables with assumed normal distributions (age at inclusion, CERAD WLT learning & recall T-scores, VOSP silhouettes T-score and TMT A & B T-scores, and COWAT T-score), between group differences were compared using analysis of variance (ANOVA). For continuous variables with non-normal distributions (MMSE and Clock drawing test), group differences were assessed using Mann-Whitney U tests. In addition, group differences in educational level being an ordinal variable, were also measured using a Mann-Whitney U test.

3.11.2 Paper II.

Differences in CSF biomarkers, MTL volumes, cognitive tests and demographics were assessed between clinical groups (APOE-$\varepsilon$4- or APOE-$\varepsilon$4+ controls, SCD and MCI groups with amyloid plaques). For variables with normal distributions, One-way ANOVAs with planned comparisons were performed. For non-normal distributions, the Kruskal-Wallis test with Dunn's nonparametric pairwise post hoc test were performed. For MTL volumes, ANOVA analyses were performed on standardized residuals after covariate regression correction for age, gender, and MRI scanner model. To compare levels of CSF neurogranin, CSF BACE1, and Ng/BACE ratio score to groups derived from the A/T/N classification.
scheme, one-way ANOVAs with post hoc Bonferroni corrections were performed. The relationships between CSF biomarkers and cognitive tests at baseline were assessed using simple regression models with age-adjusted T-scores as dependent variables. However, for MMSE, a multiple linear regression model controlling for age was used. The relationships between biomarkers and MTL volumes were assessed using multiple regression analyses controlling for effects of age, gender, and MRI scanner model. Effect sizes for the overall regression models are provided ($R^2$). CSF $\text{A}\beta_{1-42}$ was used as core selection criteria in the study design and was omitted as a predictor from baseline regression analyses with cognitive tests and MRI variables. However, CSF $\text{A}\beta_{1-42}$ was assessed as a predictor of cognitive change at 2-year follow-up. As CSF p-tau and t-tau demonstrated collinearity (variance inflation factor > 7), only CSF t-tau was included in our regression models. To measure individual change in cognitive scores between baseline and 2-year follow-up, individual follow-up scores were subtracted from baseline scores. The resulting score was used to predict cognitive changes from baseline CSF biomarkers using linear regression models. For the CERAD WLT, we used the normative performance of the DDI cohort control group (Fladby et al., 2017) to calculate T-scores following findings in paper I which showed that published norms from Sotaniemi et al. (2012) did not match the younger and more educated DDI cohort.

3.11.3 Paper III.

First, CERAD WLT performance in the healthy control group was assessed by fitting multiple regression analyses with age, gender and years of education as predictors. In addition, non-linear effects of age (i.e. improving CERAD WLT performance at younger age, and declining with older age) and a potential between-cohort bias between DDI and Trønderbrain cohorts were investigated. However, no non-linear relationships or cohort bias
were found. Thus, only linear terms were included in the final models. Overall estimates of
the regression models (adjusted $R^2$, $F$-value, $p$-value), and relative contributions for individual
predictors ($\beta$, partial $R^2$, $p$-value) were reported. Due to a marked ceiling effect, The CERAD
WLT recognition subtest did not produce a normal distribution of test scores required for the
regression-based norming procedure. However, our data indicate that age and gender had the
strongest demographic influence on test performance. Thus, cumulative percentiles of
recognition subtest for geriatric (65 – 80 years) and non-geriatric (40 – 64 years) populations
split by gender were provided.

Then, regression-based norms for CERAD learning and recall subtests were developed using
the following stepwise procedure: 1) The control groups raw test scores were normalized by
retrieving the cumulative frequency distribution of both measures. The resulting distribution
was converted into a standard scaled score with a mean of 10 and a standard deviation of 3. 2) The
resulting scaled scores were regressed on age, gender and education, and the regression
model parameters, including regression coefficients were retrieved. Plots of standardized
residuals predicted values were assessed to ensure that the assumption of homoscedasticity
was not violated, and normality of the residuals was checked visually with Q-Q plots. 3) To
derive normative information, the multiple regression equations derived from this analysis
was used to compute a persons predicted scaled score [intercept + individual age(coefficient
for age) + individual years of education(coefficient for years of education) + individual
gender(coefficient for gender)]. A person’s expected normal scaled score, derived from the
healthy control group’s normalized scaled score distribution, was then subtracted from the
regression equation predicted scaled score. The resulting discrepancy score was then divided
by the standard deviation of healthy control group’s residuals (from the regression analysis
described above) to yield a standardized z score, which can then be converted to a $T$ score.
Lastly, demographically adjusted T scores for the CERAD WLT learning and recall subtests were calculated for the DDI MCI group (n=168). Multiple regression models with age, gender and years of education as predictors were then fitted to the DDI MCI group’s T score distributions to confirm adequate adjustment of demographic variables in an independent sample.

4 Summary of results

4.1 Paper I

Title: Screening for Alzheimer's Disease: Cognitive Impairment in Self-Referred and Memory Clinic-Referred Patients.

Aims: To investigate recruitment source bias in self-referred and memory-clinic referred patient cohorts to reveal potential differences in cognitive performance and demographics in participants with SCD and MCI.

Methods: We included 431 participants 40 – 80 years old. Participants were classified as controls (n=132) or symptom group (n=299). The symptom group comprised of subjective cognitive decline (SCD, n=163) and mild cognitive impairment (MCI, n=136). We compared cognitive performance and demographics in memory clinic-referrals (n=86) to self-referred participants responding to advertisements and news bulletins (n=179). Participants recruited by other means were excluded from analysis (n=34).

Results: At symptom group level, we found significant reductions in cognitive performance in memory clinic-referrals compared to self-referrals. However, significant reductions were only found within the MCI group. We found no differences in cognitive performance due to recruitment within the SCD group. The MCI group was significantly impaired compared to controls on all measures. Significant reductions in learning, and executive functions were also found for the SCD group.
Conclusion: Regardless of recruitment source, both the SCD and MCI groups showed reduced cognitive performance as compared to controls. Differences in cognitive impairment for memory clinic-referrals compared to self-referrals were only found within the MCI group.

4.2 Paper II

Title: Cerebrospinal fluid neurogranin/β-site APP-cleaving enzyme 1 predicts cognitive decline in preclinical Alzheimer's disease.

Background/Aims: Increased CSF Ng/BACE1 ratio may reflect synaptic affection coupled to synaptotoxic Aβ oligomers in AD. The aim of this paper was to investigate if CSF Ng/BACE1 ratios are increased in SCD and MCI cases with amyloid plaques as compared to controls and if increased Ng/BACE1 ratio relates to baseline MTL volumes, baseline cognitive performance and cognitive decline at follow-up. Additionally, we investigated if healthy at-risk APOE-ε4 carriers would also show increased CSF Ng/BACE1 ratios as compared to non-carriers.

Methods: Established CSF AD biomarkers (Aβ1-42, t-tau and p-tau), and the CSF synaptic markers Ng, BACE1 and Ng/BACE1 levels were compared between cases with SCD (n = 18) and MCI (n = 20) both with amyloid plaques and healthy controls (APOE-ε4+, n = 16; APOE-ε4-, n = 20). Regression analyses were performed between cerebrospinal fluid levels, baseline hippocampal and amygdala volumes, and pertinent cognitive measures (memory, attention, Mini Mental State Examination [MMSE]) at baseline and after 2 years.

Results: APOE-ε4- and APOE-ε4+ control groups had equal levels of all CSF biomarkers. No differences in AD biomarkers were found between the SCD and MCI groups. While no significant differences in CSF Ng or BACE1 between groups were found, CSF Ng/BACE1 levels were equally elevated in both SCD and MCI compared to healthy controls. Higher CSF Ng/BACE1 ratio was the only CSF biomarker associated with lower baseline hippocampal
and amygdala volumes corresponding to lower baseline memory functions, attention, and MMSE. Increased CSF Ng/BACE1 ratios also predicted decline in MMSE and memory function at 2-year follow-up.

**Conclusions:** CSF Ng/BACE1 ratios were equally increased in SCD and MCI cases with amyloid plaques, related to baseline MTL volumes and cognitive performance and predicted cognitive decline at follow-up. Importantly, increased CSF Ng/BACE1 ratio in preclinical SCD cases may reflect synapse affection, which have yet to reach the threshold for clinical impairment. Thus, early synapse affection, guided by the CSF Ng/BACE1 ratio, could be a target for early intervention.

### 4.3 Paper III

**Title:** Demographically adjusted CERAD wordlist test norms in a Norwegian sample from 40 to 80 years.

**Background/Aims:** The CERAD WLT is a widely used test in dementia research. However, culturally adapted and demographically adjusted test norms for younger ages are lacking. The aim of this paper was to investigate normative CERAD WLT performance in healthy Norwegian speaking participants and provide demographically adjusted test norms for ages 40 – 80 years.

**Method:** Normative influences of age, gender and years of education on CERAD WLT test performance were estimated using regression analyses in healthy controls aged 40 – 80 years (n=227) from the Norwegian DDI (n=168) and Trønderbrain (n=59) cohorts. Then, a regression-based norming procedure was used to develop demographically adjusted norms for the CERAD WLT. In order to evaluate normative performance, we applied the norms to an independent sample of individuals previously diagnosed with mild cognitive impairment (MCI, n=168) and performed multiple regression analyses to evaluate adjustment of pertinent
demographics.

**Results:** CERAD WLT norms adjusted for effects of age, gender and educational level are proposed. The norms successfully adjusted for effects of age, gender and education in an independent sample of Norwegians with MCI.

**Conclusion:** This paper offers demographically adjusted norms for the CERAD WLT for ages 40 through 80 years based on a Norwegian sample. To our knowledge, this is the first normative study of this test to offer demographically adjusted norms for this age span.

5 Discussion

5.1 Summary of findings

This thesis aimed to investigate the role of SCD as a preclinical stage of AD and sought to improve early detection of at-risk individuals by investigating a potential recruitment source bias in participant inclusion of SCD, develop more sensitive test norms for episodic memory performance and investigate a new CSF biomarker of putatively AD-coupled synapse affection in SCD and MCI with amyloid plaque pathology. In paper I, we found that while there was a general bias of worse cognitive performance in memory clinic referrals, results were only statistically significant for MCI cases. However, findings from this paper have generated new hypotheses that could help delineate benign SCD from SCD due to AD, which are currently being investigated in the DDI study. This study also revealed the need to establish new test norms for the CERAD WLT. Norms were ultimately developed in paper III and found to successfully adjust for demographic influences in an independent sample of MCI cases. In paper II, the CSF Ng/BACE1 ratio was found to be increased in both SCD and MCI cases with amyloid plaques. Increased ratios were related to reductions in hippocampal and amygdala volumes, corresponding to impairments in learning and memory at baseline and predicting future cognitive decline at 2-year follow-up.
5.2 Paper I

**MCI inclusions from memory clinics are at higher risk, or later stage of disease development**

In paper I, we showed that memory-clinic referred MCI cases performed worse on cognitive tests compared to self-referred individuals. These findings generally support the notion that inclusion from memory clinics recruit individuals who are at higher risk of conversion to dementia (Farias et al., 2009; Roh et al., 2016) or who may be farther along the disease trajectory than participants recruited through other means (Brodaty et al., 2014; Whitwell et al., 2012). Moreover, the MCI participants recruited through memory clinics, while more cognitively impaired, were also younger, and could represent an earlier onset, or more aggressive form of pathology than found in the older self-referred sample. Indeed, Fladby et al. (2017) analyzed the CSF AD biomarker distributions of the DDI cohort and found that the memory clinic sample showed higher prevalence of pathological CSF AD markers and higher rates of APOE-e4 carrier status, possibly mirroring the lower cognitive performance found in the present study. These findings are in line with previous reports showing higher risks in terms of genetic risk factors (Brodaty et al., 2014), higher presence of AD-type pathology (Schneider, Aggarwal, Barnes, Boyle, & Bennett, 2009) or more aggressive forms of pathology (Whitwell et al., 2012). However, the memory clinic-referred MCI cases in our sample had a lower educational level than their self-referred counterparts. Educational level is associated with cognitive reserve (Valenzuela & Sachdev, 2006), thus lower cognitive performance in this group may also to a certain degree be confounded with a lower ability to compensate for brain pathology compared to the self-referred group.

**SCD inclusions from memory clinics may be at higher risk**

No significant differences in demographics or cognitive performance due to recruitment bias
were found within the SCD group. However, although not reaching the level of statistical significance, the data showed a trend towards both subtle lower performance and lower educational level in memory clinic-referred SCD cases compared to self-referrals. The lack of statistical significance for this result may be due to a small sample size (memory clinic-referred SCD cases (n=40)). Moreover, we did find an overall significant difference in cognitive performance at symptom group level (SCD+MCI) beyond what was shown by the MCI group alone. This suggests that although the differences are small, SCD cases recruited from memory clinics may represent a cognitively more impaired group than self-referred SCD cases. In addition, the SCD group, regardless of recruitment source, performed worse on key cognitive domains associated with AD such as learning and executive functions, as well as a general decline in overall cognitive screening performance (MMSE) compared to controls. Although observed effect sizes were small, these findings support the notion that SCD could be a symptom of awareness of subtle cognitive decline witnessed by small declines in cognitive performance, while still performing within limits of normal variations (Jessen et al., 2014). As previously noted, the Fladby et al. (2017) biomarker study has also confirmed that the SCD group in DDI cohort harbors higher rates of CSF amyloid pathology and APOE-e4 carriers as compared to controls, possibly mirroring the findings of our study. Taken together, these results support SCD as an important risk factor for AD.

*Increased depressive symptoms caused by increased awareness of SCD?*

Interestingly, a relative increase in depressive symptoms measured by the GDS 15 in the memory clinic-referred SCD cases compared to self-referrals was observed (data not shown). However, the observed increase in symptoms was not above the suggested cut-offs for clinical depression at group level (Marc, Raue, & Bruce, 2008). This is not a surprising finding since severe psychiatric illness, including major depression, is a core exclusion criterion in this
study. However, this may not be the case in all study designs investigating SCD cases.

Accordingly, recruitment from memory clinics may lead to inclusion of a higher percentage of clinically depressed individuals. The role of depressive symptoms in SCD and preclinical AD is however unclear [12]. A study by Perrotin et al. (2016) comparing SCD cases recruited from memory clinics and community sample, showed a significant reduction in gray matter volume related to AD pathology in the memory clinic group. The authors concluded that medical help seeking and increased depressive symptoms were related to this volume reduction and pointed out an increased affective burden as a potential part of prodromal AD. Conversely, Heser et al. (2013) found that depressive symptoms were fully mediated by subjective memory impairment worry, suggesting that depressive symptoms were caused by an increased awareness of subjective decline, explaining levels of depressive symptoms in individuals with subjective cognitive complaints. This latter point raises an important question. Are all persons presenting with SCD to their GP always referred to memory clinics?

*Are all SCD cases seeking medical help referred to memory clinics?*

While our findings suggest that recruitment source affects clinical characteristics of preclinical cohorts and should be taken into consideration, subjective memory impairment worry may be an important risk factor in the SCD group leading to memory-clinic referral. While SCD in general may often be a benign symptom (Bassett & Folstein, 1993; Hessen et al., 2017), worried individuals with SCD have an increased risk of developing objective cognitive decline (Jessen et al., 2014; Rabin et al., 2012; Reisberg & Gauthier, 2008). However, patients who report SCD to their GP may not always be referred to a memory clinic for assessment (Jenkins, Tales, Tree, & Bayer, 2015). Increased depressive symptoms could be caused by an increased awareness of SCD, rather than indicating a clinical depressive state (Heser et al., 2013) and subsequently prompt the individual to seek medical help. As not all
SCD cases seeking help are referred to memory clinics, some of the self-referred cases could indeed have a history of seeking medical help due to SCD. The DDI CRF includes questions of prior medical help seeking for persons recruited by self-referral and may be an important factor initially underemphasized when conducting this study. We are therefore currently investigating the role of worry and history of medical help seeking among SCD cases within the DDI study with regards to both biomarkers, demographics and cognitive impairment. Results from the current and future studies are important not only in the selection of at-risk participants for prospective research studies, but are also clinically relevant as they may inform general practitioners about risk-factors for SCD due to AD.

Methodological considerations and study limitations in Paper I

Some methodological considerations and limitations for paper I need to be addressed. First, due to geographic differences in Norway, the availability of memory clinics may differ. This could lead to a biased inclusion of memory clinic-referrals living in, or near city centers where the university hospitals are located. This may also influence the rate of which SCD cases are referred by GP to memory-clinic assessment. Second, while we at the time of the study did not include the use of biomarker evidence to further characterize selection bias, this was addressed by Fladby et al. (2017) in a parallel paper and results are included in the discussion above. Third, a general limitation in the DDI study worth mentioning is a trade-off effect due to the inclusion of younger middle-aged adults (40 – 80 years). While this offers an optimal design to capture preclinical AD and track disease development through longitudinal change, the current study was limited to a cross sectional comparison. These inclusion criteria thus lower the mean age and increase variability in the sample and may lead to dilution of AD prevalence in both SCD and MCI samples in cross sectional analyses of the DDI cohort.
Fourth, a point could be made for employing post-hoc correction for multiple testing in this paper. However, since relatively few comparisons were made with regards to recruitment source, there is a relatively low chance of increased rate of false positive discoveries (Bender & Lange, 2001). Lastly, an important incidental finding from this paper, was that the use of Sotaniemi et al. (2012) CERAD WLT normative dataset may be unfit for the DDI cohort. These norms are based on a sample that is on average 10 years older and less educated than the DDI cohort. This may in some cases result in an uncertain classification of MCI and SCD. This finding ultimately led to the development of new regression-based norms for the CERAD WLT in paper III.

5.3 Paper II

*Increased CSF Ng/BACE1 is associated with AD related MTL reductions and corresponding memory deficits and predicts future cognitive decline*

In paper II, we showed that CSF Ng/BACE1 levels were equally increased in both Aβ+ MCI and SCD groups compared to controls (figure 2). No significant group differences were found for either CSF Ng or BACE1, when measured separately. Moreover, no differences in CSF biomarker levels emerged between *APOE*-ε4+ and *APOE*-ε4- controls. These results suggest that synapse affection may be coupled to the presence of established amyloid pathology in both SCD and MCI cases. Importantly, we found that increased CSF Ng/BACE1 ratios were the only biomarker associated with reduced baseline hippocampal and amygdala volumes in our sample (figure 3). Concordantly, increased CSF Ng/BACE1 ratio was also the only biomarker associated with poorer baseline performance in both baseline CERAD learning and memory recall (figure 4), as well as attention/psychomotor speed (TMT-A), and global cognitive function (MMSE).
Figure 2. CSF Ng/BACE1 ratio (A), CSF Ng (B) and BACE1 (C) levels between groups. Abbreviations: Ctr = Controls, APOE-ε4+/−; Apolipoprotein E4 allele positive or negative, Aβ+ = CSF amyloid pathology. SCD = subjective cognitive decline. MCI = mild cognitive impairment. Horizontal brackets showing contrast comparisons for CSF Ng/BACE1 only (A). Significant results (p<.05) or non-significant results (n.s.) are shown.
When analyzing available 2-year follow up cognitive scores, we showed that lower baseline CSF Ng/BACE1 ratios predicted practice effects in the CERAD learning subtest at follow-up (i.e., showing improved performance), and increasing ratios predicted less improvement and finally a decline in CERAD word list learning ability (figure 4). This relationship was also shown for CSF Ng measured separately, supporting previous findings (Portelius et al., 2015;
Tarawneh et al., 2016). While a similar result was obtained with CSF t-tau as a baseline predictor, an inspection of the scatterplot indicated that the regression model may have been biased by a few subjects with extreme baseline CSF total tau values (figure 4). This result suggests that the subjects with high baseline measures of neuronal degradation (CSF t-tau) may be at a more advanced stage of disease development and therefore show a steeper cognitive decline. This is in line with findings linking markers of neuronal degradation to disease severity (Sämgård et al., 2010). In contrast, CSF Ng/BACE1 may represent synaptic loss that is more closely tied to smaller increments of cognitive decline along the early Alzheimer’s trajectory, which may precede markers of significant neuronal degradation. This could explain why only the CSF Ng/BACE1 ratio was related to baseline learning and memory function in our sample, possibly due to early synaptic loss in the hippocampus where neurogranin is highly expressed (Higo et al., 2004). Moreover, while higher CSF Ng/BACE1 was related to lower MMSE at baseline and decline at follow-up, CSF Ng/BACE1 was predominantly related to CERAD learning and memory recall. The MMSE contains word-list memory items, and the observed relationship could be influenced by this shared measure. Interestingly, TMT-A, a measure of psychomotor speed and attention was inversely related to CSF Ng/BACE1. This is in accordance with previous investigations showing that performance on the TMT-A is related to amyloid load in SCD cases, and mixed samples of MCI and healthy subjects (Duara et al., 2013; Loewenstein et al., 2016). To our knowledge, this is the first study showing that the Ng/BACE1 ratio is related to memory deficits and reduced MTL volumes in Aβ-positive preclinical cases and that CSF Ng/BACE1 is significantly increased relative to controls in amyloid-positive subjects with SCD.
CSF Ng/BACE1 ratio may be an early marker of synapse loss due an Aβ-coupled disease mechanism and point to possibilities for early intervention

BACE1 and neurogranin have predominantly pre (Del Prete et al., 2014; Sun & Roy, 2018) and post-synaptic roles, where neurogranin in particular is linked to the dendritic spine NMDA Ca²⁺-Calmodulin second messenger complex (Diez-Guerra, 2010). Presynaptic BACE1 cleavage of AβPP is a rate-limiting step in the production of the aggregation prone...
Aβ₁₋₄₂ species (Das & Yan, 2017), and Aβ oligomers have shown to accumulate at synaptic terminals in AD where it may disrupt postsynaptic NMDA receptors, leading to Ca²⁺ dyshomeostasis and spine degeneration (Alberdi et al., 2010; Alzheimer's Association Calcium Hypothesis, 2017; Zhang et al., 2016). As neurogranin is expressed in dendritic spines, elevated CSF concentrations in AD may reflect this process. Thus, the release of CSF neurogranin relative to the activity of BACE1 measured in CSF concentrations of this enzyme (i.e. the Ng/BACE1 ratio), may indicate a post-synaptic Aβ-linked disease mechanism, and hence better reflect AD-related synaptic degradation. The pathogenesis of AD involves degradation of the medial temporal lobe structures (C. R. Jack et al., 1997; Poulin, Dautoff, Morris, Barrett, & Dickerson, 2011) where neurogranin is highly expressed (Higo et al., 2004). Thus, the selective increases in CSF concentrations of Ng observed in AD (Wellington et al., 2016) may occur as consequence of degradation of these structures. Hippocampal and amygdala volume reductions were indeed significantly related to higher CSF Ng/BACE1 levels in our study, which suggest that the CSF Ng/BACE1 ratio may relate to synapse loss in these regions. Moreover, while CSF Ng/BACE1 was similarly increased in the Aβ+ MCI and SCD groups, the latter still performed within the normal range on cognitive tests. This may reflect an active disease state of progressive synaptic loss, which has yet to reach sufficient loss needed for clinical impairment and may offer possibilities for intervention. Interestingly, Insel et al. (2017) recently demonstrated that subtle memory decline, corresponding to cortical atrophy and hypometabolism in the temporal and medial temporal regions may begin several years before biomarkers of amyloid plaque pathology become positive. However, this was not shown for the parietal cortex or other lobes, where the spread of pathology was evident only after established plaque pathology, corresponding to declines in global cognition. This suggests a temporal sequence where early pathological changes could be tied to synapse affection preceding substantial neuronal loss and tangle formation seen at later stages. The
formation of Aβ oligomers precede parenchymal plaque deposition and show synaptotoxic properties (Alberdi et al., 2010; Alzheimer's Association Calcium Hypothesis, 2017; Hong et al., 2016; Zhang et al., 2016). Thus, if Aβ oligomers are responsible for early synapse loss in AD, CSF Ng/BACE1 ratios may increase in the years preceding plaque formation. Importantly, NMDA antagonists have been suggested as protective in AD (Wang & Reddy, 2017). If our hypothesis is confirmed, such intervention guided by early CSF Ng/BACE1 increase might be useful.

**Presence of APOE-ε4 allele may enhance oligomerization of Aβ peptides**

While APOE-ε4 allele carrier status was included as a predictor of both MTL volumetry and cognition in this study, no significant associations were found. However, a large majority of the Aβ+ SCD and MCI cases (28 of 37) had at least one APOE-ε4 allele and APOE-ε4 carriers with amyloid plaques had higher CSF Ng/BACE1 compared to non-carriers with plaques (data not shown). In this scenario, enhanced synaptotoxic polymerization of Aβ-peptides in APOE-ε4 SCD and MCI carriers could have a more rapid synaptic loss due to increased levels of synaptotoxic Aβ fibrils (Alberdi et al., 2010; Huynh et al., 2017; Sanan et al., 1994). However, while APOE-ε4 could enhance CSF Ng/BACE1 related pathology through its interaction with Aβ (Alberdi et al., 2010; Huynh et al., 2017; Sanan et al., 1994), a larger material with more APOE-ε4 negative and Aβ+ SCD and MCI cases is needed to establish ε4-allelic effects. At the time when this study was conducted, CSF Ng and BACE1 levels were only available for a subset of DDI participants, selected in accordance with the study design for this paper. However, CSF analyses were completed for the entire DDI cohort.
in early 2019 and analyses of \textit{APOE}-\(\epsilon\)4 allelic effects on CSF Ng/BACE1 related pathology are currently being investigated.

\textit{Alternative hypotheses for increased CSF Ng/BACE1: the tau hypothesis and the role of the innate immune system in AD}

We found that CSF Ng/BACE1 ratios increased with A/T/N classified AD biomarker severity, (i.e. moving from normal CSF towards amyloid plaques combined with markers of neurodegeneration and neurofibrillary tangles) (C. R. Jack et al., 2018). In addition, an increase was also observed for both CSF BACE1 (Barao et al., 2013) and Ng (De Vos et al., 2016), when measured separately. These results support previous findings indicating a link to neurodegeneration.

However, these findings also point to an important question due to a central limitation in our study. As we did not include A\(\beta\)-negative SCD or MCI cases, our findings do not conclusively support the hypothesis that increased CSF Ng/BACE1 ratio is linked to amyloid pathology. It has been shown that the spread of tau pathology (neurofibrillary tangles) is more closely linked to clinical progression in AD than amyloid pathology (Bejanin et al., 2017) and may lead to cognitive deficits through a variety of mechanisms, including neurodegeneration. Moreover, impairments in A\(\beta\)PP metabolism have shown to induce axonal and synaptic defects independently of the buildup of beta-amyloid (Rodrigues, Weisssmiller, & Goldstein, 2012). Kametani and Hasegawa (2018) argue that this may cascade into the propagation of pathological tau and neurofibrillary tangle formation. Thus the spread of tau may cause synapse degeneration and neuronal loss independently of amyloid deposition. The spread of tau, rather than beta-amyloid, may be the main cause of AD. However, the authors also note that neuroinflammation caused by amyloid deposition may further affect the progression of tau pathology (Kametani & Hasegawa, 2018). However, alternative views exist, and new
developments also points to a central role of microglia in Alzheimer’s related synapse loss (Rajendran & Paolicelli, 2018). Complement mediators such as C1q and C3 are highly increased with amyloid deposition in experimental studies (Reichwald, Danner, Wiederhold, & Staufenbiel, 2009) and a recent study has shown that mice injected with of Aβ oligomers leads to upregulation of C1q and C3 levels, which in turn promote microglia removal of synaptic connections by phagocytosis. Furthermore, it was shown that synapse loss in the hippocampus was rescued in mice treated with an anti-C1q antibody (Hong et al., 2016). It has also been shown that AD mouse models depleted of C3 reduces synapse loss and promotes cognition regardless of continued amyloid accumulation (Shi et al., 2017). These studies suggest a microglia complement-dependent pathway of synapse loss in AD due to effects of Aβ oligomers. This could putatively lead to the observed CSF Ng/BACE1 ratio increases in Aβ+ SCD and MCI cases in our study. While more work is needed to further delineate the precise sequence of pathological events and associated mechanism, CSF Ng/BACE1 ratio may be a promising biomarker for Alzheimer’s related synaptic loss owing to its strong associations to volume reductions in pertinent medial temporal lobe structures and cognitive measures in our study. These results warrant further studies investigating the role of CSF Ng/BACE1 in the AD pathogenesis, potentially reflecting synaptic pathology due to an Aβ-linked disease mechanism.

*Methodological considerations and study limitations in Paper II*

An important finding in this study, was the prominent relationship between higher CSF Ng/BACE1 ratio and reduced amygdala volume. It has been shown that amygdala atrophy is prominent in early AD, related to global illness severity, and may relate to neuropsychiatric symptoms such as anxiety and irritability (Poulin et al., 2011) and to changes in memory consolidation due to emotional arousal (Satler et al., 2007). Neuropsychiatric symptoms are
prevalent in AD (Lyketsos et al., 2002), and CSF Ng/BACE1 related synapse affection in the amygdala could putatively relate to some of the neuropsychiatric symptoms observed in AD. However, as measures of neuropsychiatric symptoms were not included in this paper, potential relationships are unknown. In 2017/2018, the DDI study established a new cohort (DDI plus) with a focus on investigating neuropsychiatric symptoms as a part of the preclinical phases of AD and other forms of dementia. Thus, in future studies, the link between CSF Ng/BACE1 related synapse loss in the amygdala and neuropsychiatric symptoms should be investigated.

As discussed above, a central limitation in this study was the omission of Aβ-negative SCD or MCI cases. In order to establish AD specificity for the CSF Ng/BACE1 ratio, including APOE-ɛ4 effects, a larger material with both Aβ+ and Aβ- SCD and MCI cases will be needed. In addition, these findings have to be interpreted cautiously due to a relatively small baseline sample size (n=74), confined to small subgroups, and the even smaller sample size with available cognitive tests at a relatively short 2-year follow-up interval (n=42). However, we are currently investigating the CSF Ng/BACE1 ratio concerning these issues owing to the completion of CSF Ng and BACE1 analyses for the entire DDI cohort in early 2019. This will yield a significantly larger material for the next round of analyses, including the investigation of the role of CSF markers for neuroinflammation and APOE-ɛ4 (Nordengen et al., 2019) with respect to synapse loss in the AD trajectory.

5.4 Paper III

Development of regression-based norms for the CERAD WLT

In paper I we discovered that the CERAD WLT norms sourced for the DDI study (Sotaniemi et al., 2012) may not be suitable due to the DDI cohort being on average 10 years younger and more educated than what these norms were aimed for. However, since the CERAD WLT was
developed for detecting MCI and dementia in geriatric populations, available norms are mostly developed for elderly cohorts (Beeri et al., 2006; Fillenbaum et al., 2005; Sotaniemi et al., 2012; Welsh et al., 1994). While Hankee et al. (2016) provide normative data for younger ages (primarily for ages 35 through 55 years), these norms would not be suitable for the DDI cohort due to insufficient coverage of older ages in the DDI cohort (40 – 80 years). In addition, while it is shown that performance on the CERAD WLT is affected by age, education and gender (Beeri et al., 2006; Heaton et al., 2004; Liu et al., 2011), these norms were only adjusted for either age or education. Lastly, these norms are based on an American sample, and norms for Scandinavian speaking countries (Danish, Swedish or Norwegian language) are lacking. Thus, in paper III, we sought to develop regression-based demographically adjusted norms for the CERAD WLT based on a Norwegian sample.

In line with previous reports, our results showed that increasing age had the strongest impact on CERAD word list performance (Sotaniemi et al., 2012; Welsh et al., 1994), followed by smaller effects of education (Beeri et al., 2006) and gender (Beeri et al., 2006; Heaton et al., 2004; Liu et al., 2011). In addition, we investigated a potential non-linear effect of age on performance (i.e. increasing memory capacity in early life superseded by a slow decline in later life). However, non-linearity was not demonstrated in our data, possibly because learning and memory capacity is fully developed or showing normal age-related decline in this age cohort (Hartshorne & Germine, 2015). While we included healthy controls from both the DDI and Trønderbrain cohorts, no between-cohort bias on performance was found. Thus, the norms were developed adjusting for age, education and gender based on the healthy controls (n=227) from both DDI and Trønderbrain cohorts.
Successful adjustment of pertinent demographics in an independent sample

A primary utility of these norms is to detect cognitive decline not caused by normal aging or expected performance differences due to gender or educational attainment. Thus, to evaluate the regression-based norms, we calculated T scores in a group of Norwegian speaking patients (n=168) aged 40 through 80 years previously diagnosed with MCI from the DDI cohort and fitted regression models to confirm that the norms reliably adjust for demographic variables when applied to an independent sample. We found that the regression-based norms successfully adjusted for age, gender and years of education in this sample. Moreover, estimated T scores in the MCI group reflected an impaired normative performance with mean scores below 1 SD compared to the healthy controls. Owing to the successful adjustment of pertinent demographics, impaired learning and memory recall on the CERAD WLT should be due to factors largely independent of normal aging, gender differences and educational level.

The CERAD WLT may be too easy for younger individuals

An important finding using the predictions offered from the regression norms was that younger people between the ages of 40 – 50, and especially women, generally do very well on this test, and the estimated normative performance for these individuals is therefore truncated and skewed. The CERAD WLT consists of only 10 words, and may therefore be too easy. Thus, in order to detect longitudinal change in cognitive proficiency due to degenerative brain disease, the CERAD WLT may not be optimal. For memory clinics and prospective research studies including younger participants, a more challenging wordlist test such as the Rey Auditory Verbal Learning Test (RAVLT) (Schmidt, 1996) may be better suited.
In line with previous reports, years of education predicted higher performance on both CERAD WLT learning and recall subtests. However, the explained variance was relatively low (about 2%) compared to gender (about 5%). The relatively low variance explained by this variable may be due to a high mean educational level in both the healthy control group (14.2 years) and in the independent MCI group (13.6 years). While these mean levels seem fairly high, they are consistent with Norwegian population statistics ("Statistics Norway," 2018), which indicate that 37.4% of Norwegians have completed upper secondary school (12-13 years) and 33.4% of the population have obtained a university degree (bachelor or higher) with more than 15 years of education in total. As such, the relatively high educational level observed in our study could represent a cultural bias, which could influence estimated normative performance on neuropsychological tests (Hayden et al., 2014; Heaton et al., 2004).

This finding raises an important question for other cognitive tests presently used in the DDI study. The cognitive screening battery presently includes the TMT A & B subtests as well as the verbal fluency measure, COWAT. Both tests use norms derived from a large American normative study published in 2004 (Heaton et al., 2004). While these norms use a similar regression-based norming procedure adjusting for several demographics, they may nevertheless be unsuited due to possible differences in educational estimates at different ages (i.e. higher mean level of education in the Norwegian sample, and educational backgrounds for elderly in 2019 may be different as compared to 2004). Thus, the American norms could be based on different relative estimates of educational influences at different ages and thus provide estimates that do not fit the expected normative performance in the Norwegian sample. This could impact normative estimates of cognitive performance and in some cases lead to misclassification of cognitively normal and impaired individuals. Consequently,
normative studies of the other cognitive tests included in the DDI study are currently in progress.

A solution for computing regression based normative scores in the clinic

While many clinicians are familiar with using conventional discrete norms, regression-based norms may not be as easy and familiar to use. In addition to providing a detailed step-by-step procedure in paper III, we have developed a free web-based intuitive normative calculator (https://uit.no/ressurs/uit/cerad/cerad-calc.html). The functionality is straight forward and intuitive, not requiring knowledge of the regression equations used to derive normative estimates. An illustration of the normative calculator is shown in figure 5.

![CERAD Word List Test Norm Calculator](https://uit.no/ressurs/uit/cerad/cerad-calc.html)

**Figure 5.** An illustration of the CERAD WLT web-based normative calculator layout

Methodological considerations and study limitations in Paper III

Regression-based norming procedures require stringent methodological criteria to be fulfilled
(Testa et al., 2009). However, when criteria are met, this method has several advantages over the conventional discrete norming approach. Since we are using the entire normative sample, regression norming allows for the adjustment of several covariates in a linear fashion, meaning that the estimation of normative performance is possible at yearly increases in age and education for both males and females. Moreover, this is achieved with a lower sample size than required by discrete norms (Oosterhuis et al., 2016). However, when assumptions of linear regression are violated (i.e. normal distribution of errors, homoscedasticity and linearity), this method may produce biased and unreliable estimates (Oosterhuis, et al., 2016). In this study, efforts were made to ensure that assumptions of homoscedasticity and normal distributions of residuals were met. As previously mentioned, non-linear effects were also assessed by accounting for non-linearity by introducing an age squared term in our regression models.

A limitation of this study regards the missing scores on the CERAD WLT recognition memory test. Also, this subtest showed a marked ceiling effect, and did not produce a normal distribution of test scores required for regression-based norming. However, our data indicate that age and gender have the strongest influence on normative performance. Thus, normative performance on this test was shown by providing cumulative percentile ranks for geriatric (≥65) and non-geriatric (≤64) age groups, further split by gender. Secondly, we did not have a complete longitudinal record of our healthy controls to verify that they remained cognitively healthy within a reasonable timeframe. Thirdly, while the regression equations will mathematically estimate age, and educational effects beyond the age and education range in this study, estimates are not reliable beyond these ranges. Lastly, an important general note on MCI cutoff criteria in the DDI study needs to be addressed. While the National Institute on Aging and Alzheimer's Association (NIA-AA) (Albert et al., 2011) recommends a cutoff criteria at between -1 and -1.5 SD below the normative mean on standardized cognitive tests,
the DDI study has opted for stringent cutoff at ≤−1.5 SD. In addition, the MMSE was used to determine MCI with a cutoff set at ≤27. However, as the MMSE is not adjusted for effects of demographics, the use of this criterion may lead to higher rates of false positive MCI. These factors could impact classification rates of SCD vs MCI within DDI. In addition, in the current thesis, all SCD/MCI classifications were made with CERAD WLT norms from Sotaniemi et al. (2012). As discussed, these norms were not suitable, and may have affected disease stage classifications. However, efforts were made in paper II to overcome this by using T scores calculated on the basis of DDI control groups performance (Fladby et al., 2017) when relating pertinent CSF biomarkers to cognitive performance. In 2019, the MMSE was dropped as a criterion of MCI diagnosis in the DDI study. Moreover, after the publication of paper III, the entire DDI cohort was restaged according to the new demographically adjusted CERAD WLT norms. Our planned normative studies for the remaining cognitive tests in the DDI battery will further serve to improve estimated of cognitive performance for future papers in DDI.

The potential use of regression-based norms to account for practice effects

An important note, not addressed in this paper, is the role of practice effects when assessing participants at follow-up (Salthouse, Schroeder, & Ferrer, 2004; Wilson, Li, Bienias, & Bennett, 2006). It has been demonstrated that not only cognitively healthy persons, but also persons diagnosed with MCI show practice effects at retest (Duff et al., 2007). Thus, a person showing no change in normative T scores between baseline and follow-up may in fact represent decline rather than cognitive stability. Participants are reassessed with the same cognitive tests at 2-year intervals in DDI. This raises an important issue, as using baseline-derived norms ignore practice effects between assessments, which can lead to underdiagnoses of MCI at follow-up (Elman et al., 2018). A potential solution is to use a regression-based
approach to estimate relative expected normative practice effects between baseline and follow-up time points within the DDI study. Similar to the regression-based norming procedure detailed in this thesis, a multiple regression model with age, gender, education as well as baseline scores (Time point 1) could be fitted to model normative performance at follow-up (Time point 2) (Duff, 2012). In the DDI study, participants are invited to follow-up examinations every 2 years. If norms accounting for practice effects are developed for several time points (e.g. 2, 4, 6 or 8 years), a linear mixed model approach may be appropriate (Salthouse et al., 2004). However, this also requires an adequate sample size of normal healthy controls at different time points. Presently it is unknown how many of our healthy controls will come for additional visits. However, if sufficient data will be available for such analysis, future normative studies in DDI should attempt to tackle this important issue.

6 Conclusions and future directions

This thesis is based on three published papers, which provide important findings to the ongoing research on preclinical AD. While we did not show significant recruitment source biases for memory-clinic referred as compared to self-referred SCD cases, our findings have generated new hypotheses. These are currently being investigated in DDI and may help distinguish benign SCD from SCD due to pathology such as AD. In addition, this work revealed the need for new test norms for the CERAD WLT better suited for the younger and more educated DDI cohort. To our knowledge, this was the first paper providing CERAD WLT demographically adjusted norms for this age range. Memory performance is a central part of AD research and sensitive and culturally adapted tools to capture normative performance differences caused by pathological processes are an important contribution to the DDI study, and possibly for the many clinicians in Scandinavia which rely on this test. This
work also pointed out the potential need to develop new demographically adjusted norms for other commonly used cognitive tests, such as the TMT A & B, which is one of the most used neuropsychological test in the Nordic countries (Egeland et al., 2016).

The Neurogranin/BACE1 ratio is a promising marker for synapse affection in AD. To our knowledge, this is the first paper demonstrating Neurogranin/BACE1 ratio synapse affection at the preclinical SCD stage, which also related to pertinent medial temporal lobe structures, memory recall deficits and future cognitive decline. This ratio may be connected to an Aβ-linked synaptic pathomechanism. If confirmed, this would point to the synapse as a nidus of early disease development in AD and could open possibilities for early intervention through NMDA receptor antagonists. However, alternate pathomechanisms putatively leading to increases in Neurogranin/BACE1 ratio need to be investigated. The precise sequence of pathological events leading to AD dementia is still unknown. However, the advances in PET imaging, CSF and blood proteomics and cognitive assessment tools, promises to further advance our understanding of AD pathology and possibilities for future intervention or prevention therapies.

The unique multimodal and longitudinal design of the DDI study holds promise for even more exciting discoveries in the next round of analyses!

7 References


Del Prete, D., Lombino, F., Liu, X., & D'Adamio, L. (2014). APP is cleaved by Bace1 in pre-synaptic vesicles and establishes a pre-synaptic interactome, via its intracellular
domain, with molecular complexes that regulate pre-synaptic vesicles functions. *PLoS ONE*, 9(9), e108576.


Jansen, I. E., Savage, J. E., Watanabe, K., Bryois, J., Williams, D. M., Steinberg, S., . . .


Jessen, F., Amariglio, R. E., van Boxtel, M., Breteler, M., Ceccaldi, M., Chetelat, G., . . .


or from a memory clinic: Differential affective and imaging correlates. *Alzheimers Dement.*


Petersen, R. C. (2016). Mild cognitive impairment. *Continuum (Minneap Minn),* 22(2 Dementia), 404-418.


Impairment Study) and CREDOS (Clinical Research Center for Dementia of South Korea). J Alzheimers Dis, 53(2), 463-473.


