UiT
THE ARCTIC UNIVERSITY OF NORWAY

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# Dementia Disease Initiation: Identifying subjective cognitive decline (SCD) due to Alzheimer's disease

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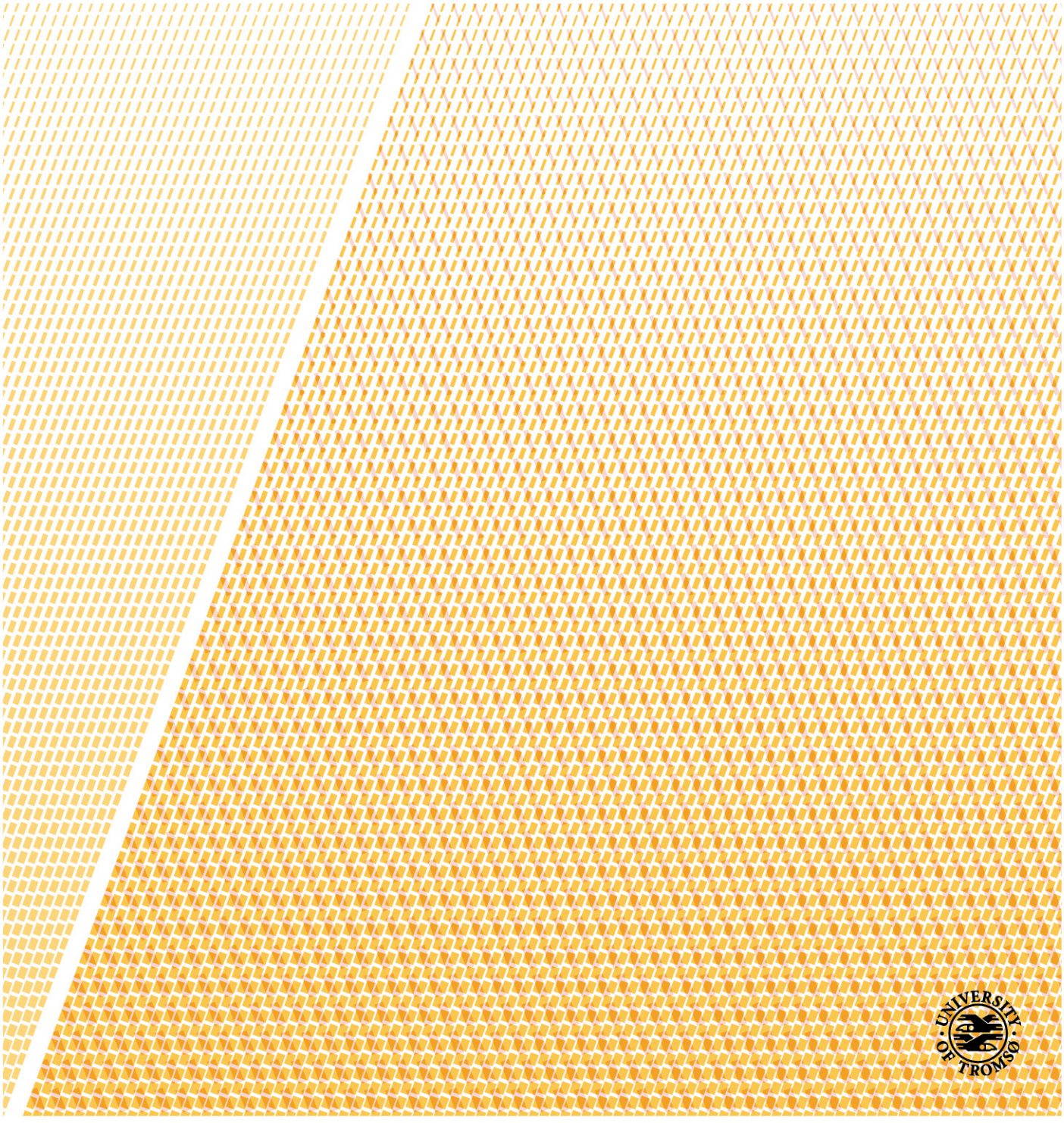
*A dissertation for the degree of Philosophiae Doctor – April 2019*



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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

Paper I: Kirsebom, B. E., Espenes, R., Waterloo, K., Hessen, E., Johnsen, S. H., Brathen, G., Aarsland, D. and Fladby, T. Screening for Alzheimer's Disease: Cognitive Impairment in Self-Referred and Memory Clinic-Referred Patients. *Journal of Alzheimer's disease: JAD*, 2017;60(4):1621-1631.

Paper II: Kirsebom, B.E., Nordengen, K., Selnes, P., Waterloo, K., Torsetnes, S.B., Gísladóttir, B., Brix, B., Vanmechelen, E., Bråthen, G., Hessen, E., Aarsland, D. and Fladby, T. Cerebrospinal fluid neurogranin/β-site APP-cleaving enzyme 1 predicts cognitive decline in preclinical Alzheimer's disease. *Alzheimer's & Dementia: Translational Research & Clinical Interventions: A&D TRCI*, 2018;(4):617-627.

Paper III: Kirsebom, B. E., Espenes, R., Hessen, E., Waterloo, K., Johnsen, S. H., Gundersen, G., Botne Sando, S., Rolfseng Grøntvedt, G., Timón, S. and Fladby, T. Demographically adjusted CERAD wordlist test norms in a Norwegian sample from 40 to 80 years. *The Clinical Neuropsychologist: TCN.* 2019;1-13

## List of abbreviations

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

## 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

(Karantzoulis & Galvin, 2011). 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 $A\beta_{1\text{-}42}$, 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 $A\beta_{1-42}/A\beta_{1-40}$ 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 $A\beta_{1-42}$ 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 4 h at 2000 $g$ for 10 min 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 $A\beta_{1\text{-}42}$, 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 $A\beta_{1\text{-}42}$ <708 for amyloid plaque pathology was determined following DDI PET [$^{18}$F]-Flutemetamol uptake studies (Kalheim, Fladby, Coello, Bjørnerud, & Selnes, 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 nor 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.

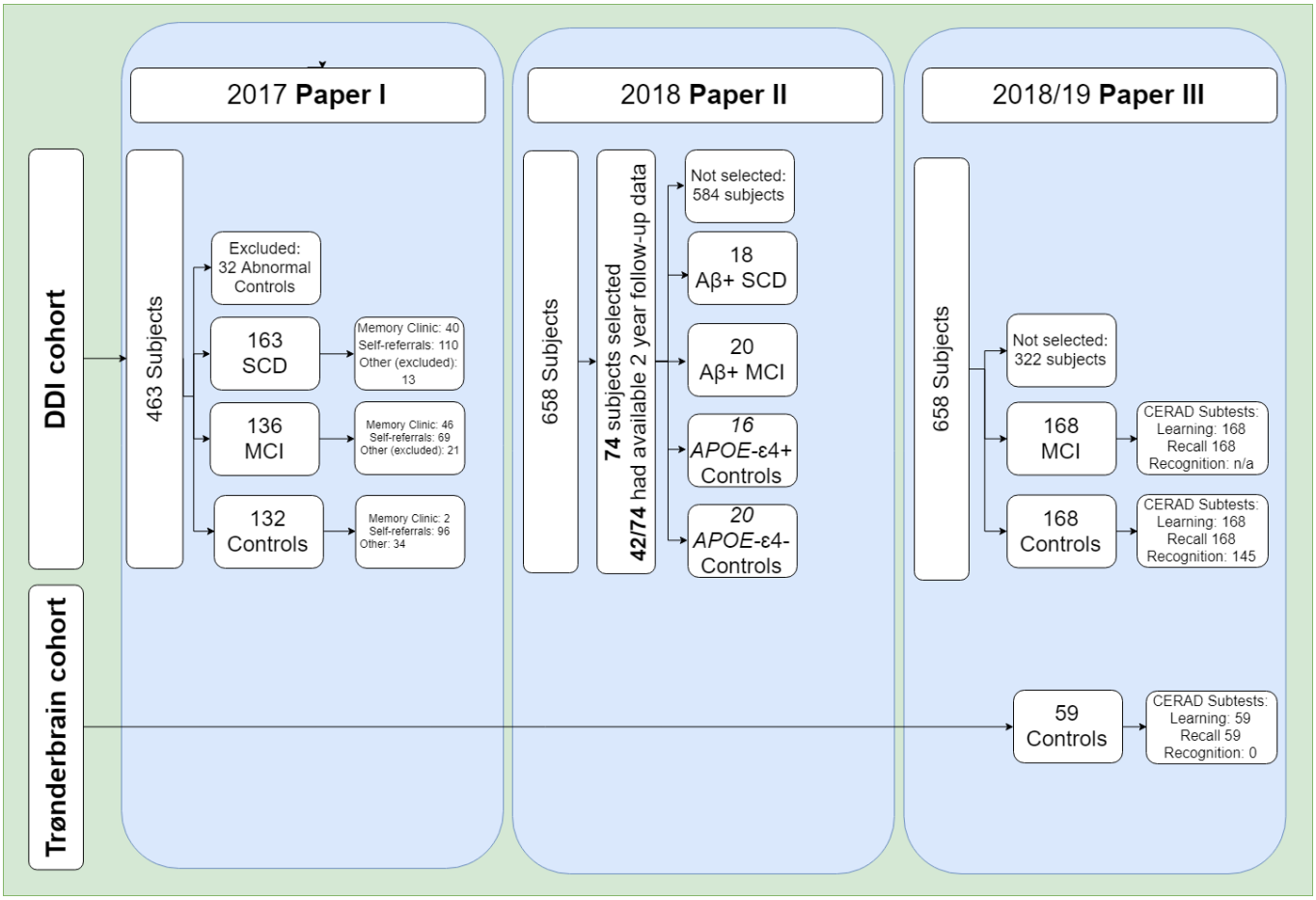


**Figure 1**. Participant selections from the DDI and Trønderbrain cohort according to papers I-III

### 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 ($\eta p^2$) 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*-ε4- or *APOE*-ε4+ 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 $A\beta_{1–42}$ 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 $A\beta_{1–42}$ 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 ($A\beta_{1-42}$, 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, =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).

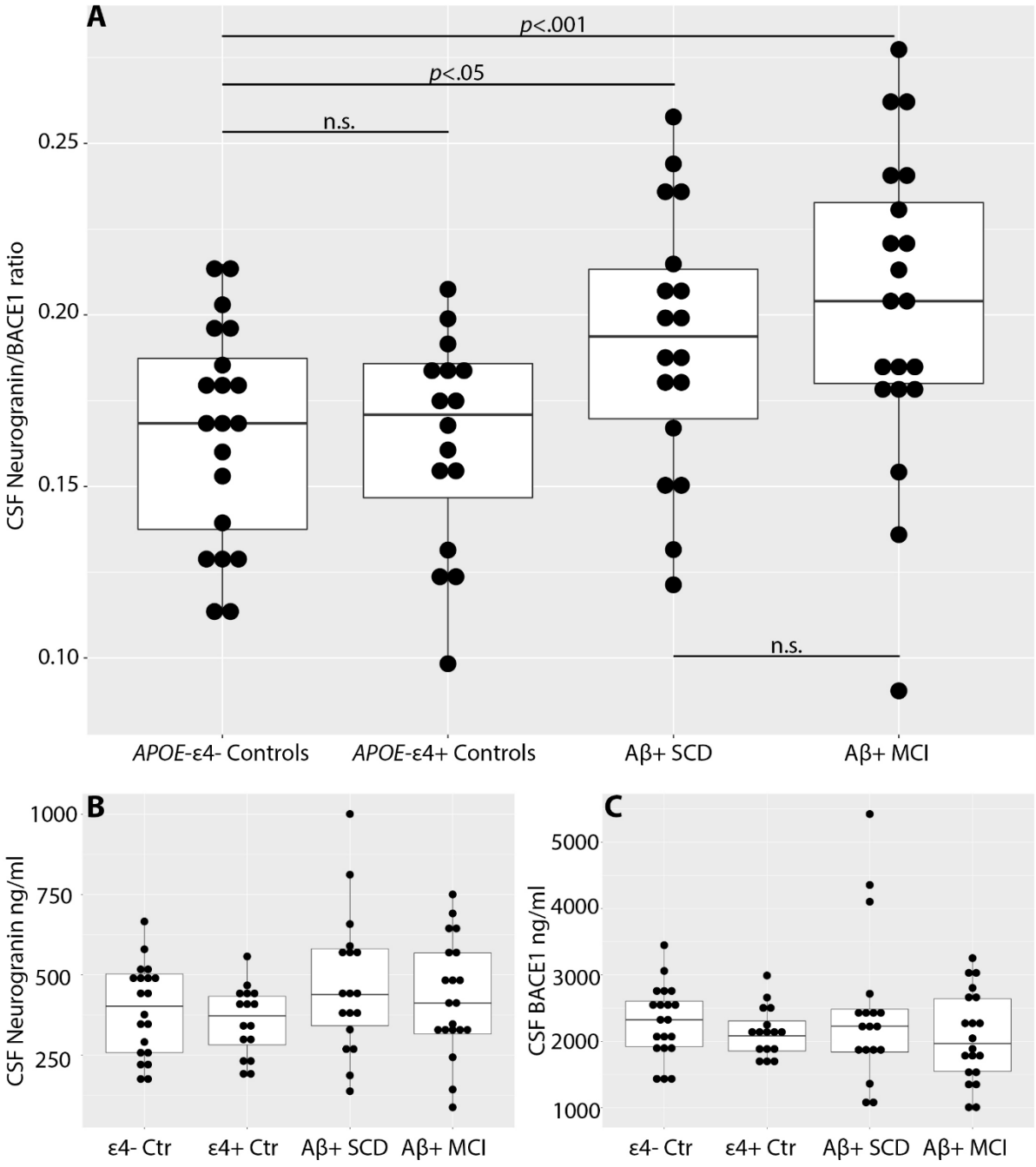


**Figure 2.** CSF Ng/BACE1 ratio (A), CSF Ng (B) and BACE1 (C) levels between groups. Abbreviations: Ctr = Controls, APOE-ε4+/-; Apolipoprotein E4 allele positive or negative, Aβ+ = CSF amyloid pathology. SCD = subjective cognitive decline. MCI = mild cognitive impairment. Horizontal brackets showing contrast comparisons for CSF Ng/BACE1 only (A). Significant results (p<.05) or non-significant results (n.s.) are shown.

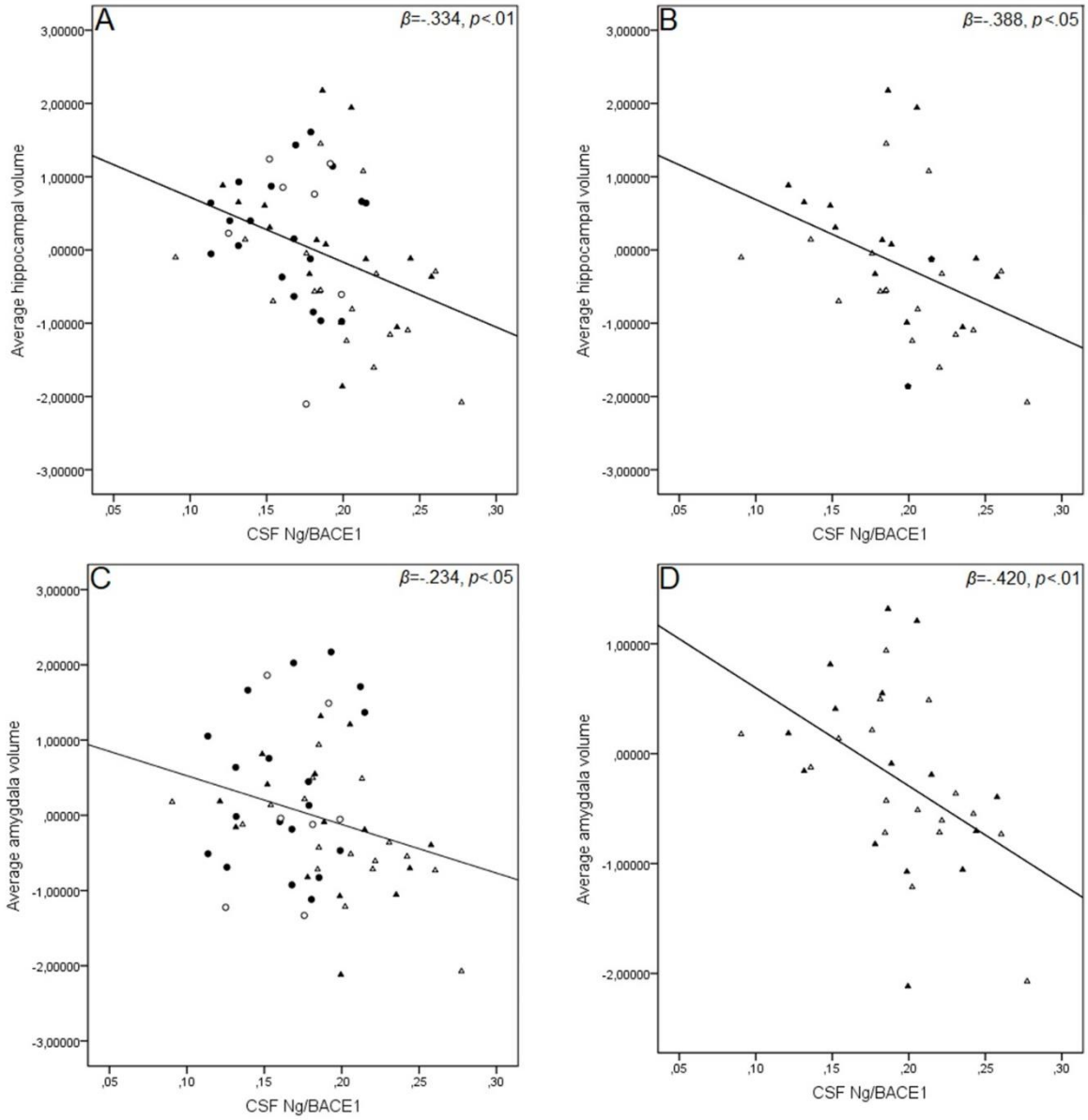


**Figure 3**. CSF Ng/BACE1 in relation to medial temporal lobe volumetry. Average hippocampal (A & B) and amygdala volumes (C & D). Medial temporal lobe volumes are adjusted for age, gender and MRI scanner variant. Open circles = APOE-ε4+ controls. Closed circles = APOE- ε4- controls. Open triangles = MCI with amyloid plaques. Closed triangles = SCD with amyloid plaques.

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.

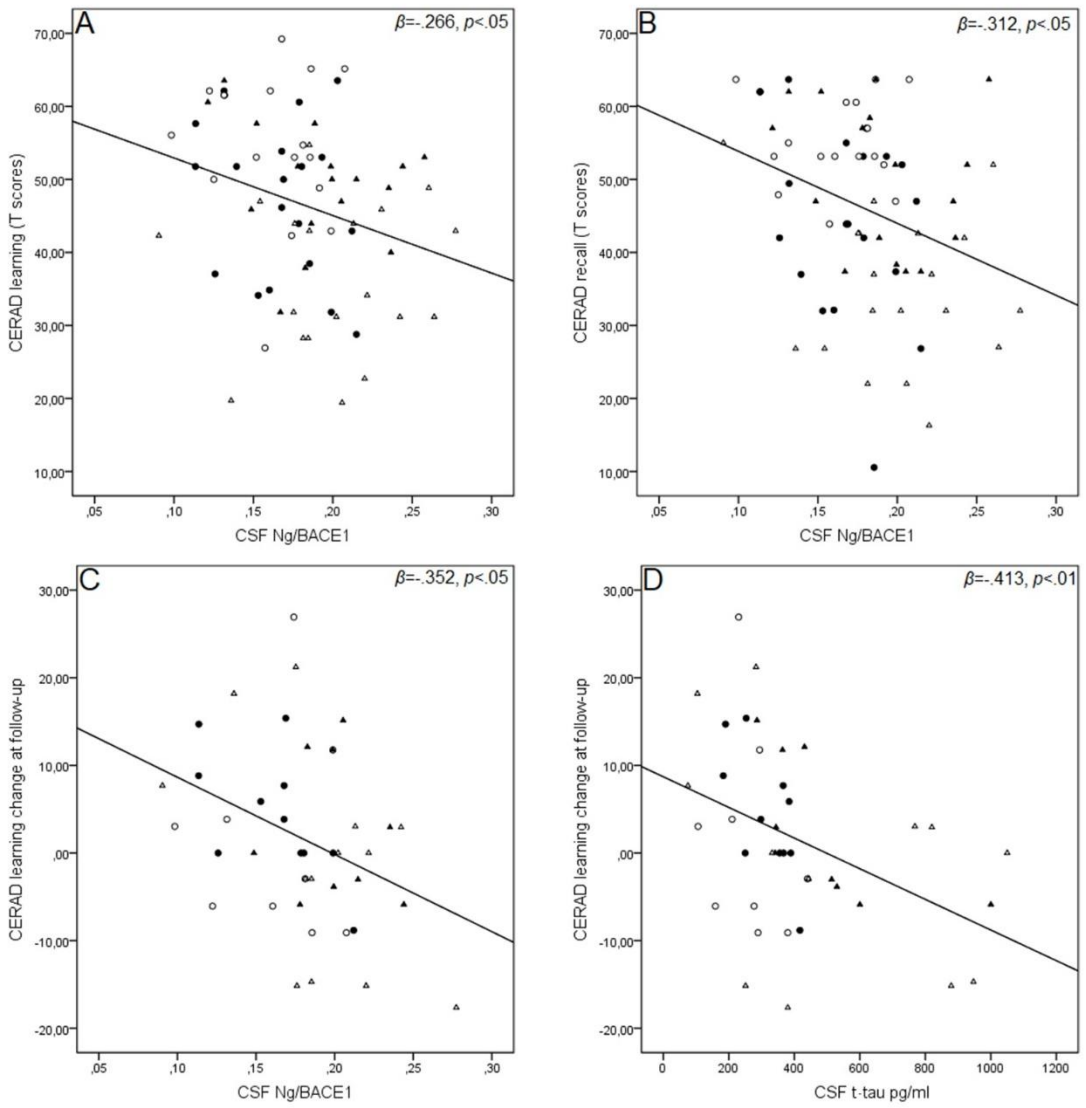


**Figure 4.** CSF Ng/BACE1 and CSF t-tau in relation to baseline and 2-year follow-up CERAD learning and memory recall tests. CSF Ng/BACE1 and baseline CERAD subtest T scores (A & B). CERAD Learning T score change at follow-up CSF Ng/BACE1 (C), CSF t-tau (D). Open circles = APOE-ε4+ controls. Closed circles = APOE-ε4- controls. Open triangles = MCI with amyloid plaques. Closed triangles = SCD with amyloid plaques. Abbreviations: CERAD = The Consortium to Establish a Registry for Alzheimer's Disease word list test.

*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 $Ca^{2+}$-Calmodulin second messenger complex (Diez-Guerra, 2010). Presynaptic BACE1 cleavage of AβPP is a rate-limiting step in the production of the aggregation prone

$A\beta_{1\text{-}42}$ 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 $Ca^{2+}$ 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 *APOE*-ε4 allelic effects on CSF Ng/BACE1 related pathology are currently being investigated.

*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 Aβ-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 AβPP metabolism have shown to induce axonal and synaptic defects independently of the buildup of beta-amyloid (Rodrigues, Weissmiller, & 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*-ε4 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*-ε4 (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.

*Cultural bias on educational level and consequences for other commonly used cognitive tests*

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*.

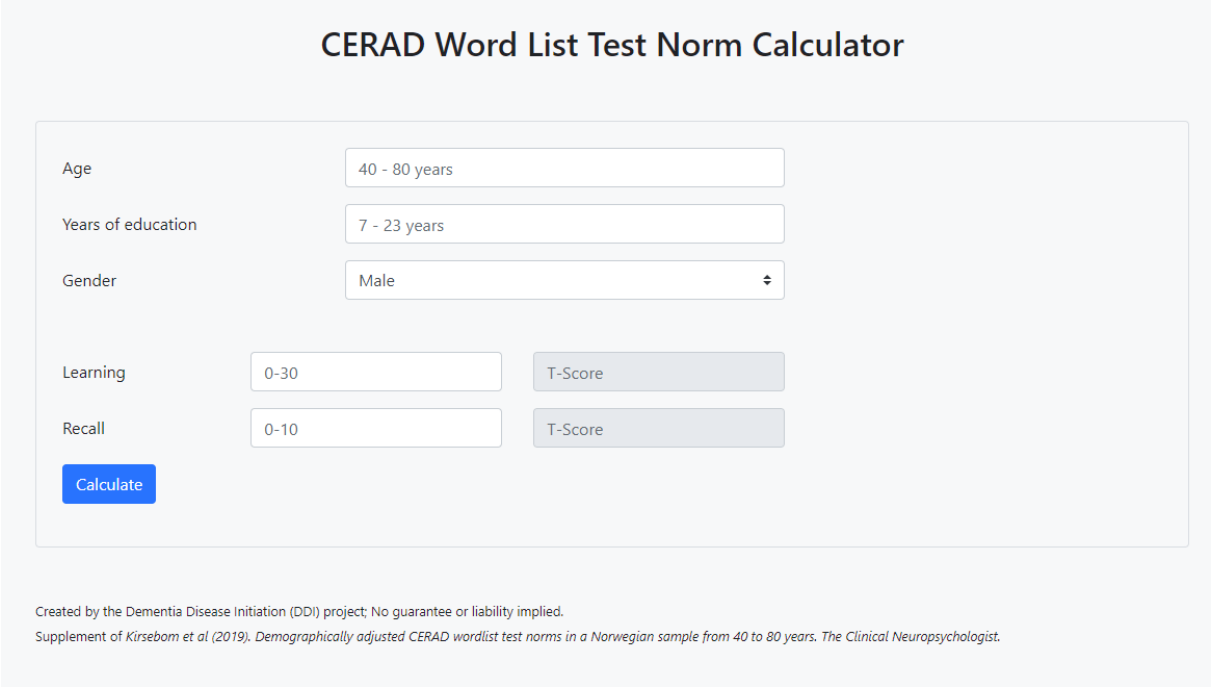


**Figure 5.** An illustration of the CERAD WLT web-based normative calculator layout

*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!

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Journal of Alzheimer's Disease 60 (2017) 1621–1631
DOI 10.3233/JAD-170385
IOS Press



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

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Handling Associate Editor: Frank Jessen

Accepted 21 August 2017

**Abstract**.
**Background:** Cognitive assessment is essential in tracking disease progression in AD. Presently, cohorts including preclinical at-risk participants are recruited by different means, which may bias cognitive and clinical features. We compared recruitment strategies to levels of cognitive functioning.
**Objective:** We investigate recruitment source biases in self-referred and memory clinic-referred patient cohorts to reveal potential differences in cognitive performance and demographics among at-risk participants.
**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, here 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.

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ISSN 1387-2877/17/$35.00 

**Conclusion:** Regardless of recruitment method, both the SCD and MCI groups showed reductions in cognitive performance compared to controls. We found differences in cognitive impairment for memory clinic-referrals compared to self-referrals only within the MCI group, SCD-cases being equally affected irrespective of referral type.

Keywords: Alzheimer's disease, cognitive dysfunction, mild cognitive impairment, patient recruitment, research subject recruitment, sampling studies, subjective cognitive decline

## INTRODUCTION

Mild cognitive impairment (MCI) is associated with an increased risk for Alzheimer's disease (AD). MCI due to AD constitutes a transitory phase between normal cognitive function and dementia [1]. Research efforts have aimed to define features and trajectories of MCI due to AD to predict conversion from MCI to AD, and to distinguish MCI due to AD from other causes of MCI such as vascular disease, early frontotemporal dementia, or early stages of dementia with Lewy bodies [1, 2]. Converging evidence from studies of at-risk cohorts and clinically normal older individuals now indicate that the pathophysiological underpinnings of AD may begin 10 to 15 years before the emergence of clinical symptoms [3]. Consequently, this has led to the proposal that AD has a preclinical phase wherein brain-compensatory mechanisms make up for early pathological changes [4]. Identifying individuals at risk for AD in the preclinical phase is a key objective [5, 6]. Future effective treatments at this level could serve to preserve or delay onset of objective cognitive decline [4, 7, 8].

A proposed target population for preclinical AD is patients with subjective experience of cognitive deficits, hypothesizing that subjective cognitive decline (SCD), i.e., with normal performance on standardized cognitive tests, may imply risk for conversion to MCI and ultimately AD dementia [6]. SCD manifests before the onset of clinical identifiable impairment, such as objective cognitive decline 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 [9–11]. A recent systematic review of subjective cognitive complaints (SCC) risk to AD/MCI progression reported that 16 out of 17 studies showed a 1.5- to 3-fold higher risk of progression in patients 59 years or older [12]. However, it should also be noted that the overwhelming majority of studies did not show progression from SCC to objective cognitive decline (MCI or Dementia) when assessed at follow-up. Bassett and Folstein [13] have shown that up to 43% of those aged between 65 and 74 years report subjective memory problems, while dementia prevalence in this age range is low. Thus, in many cases, the experience of cognitive decline is either benign, or caused by other conditions or disorders than AD. Consequently, there is a need to identify the characteristics of SCD due to AD and other disorders, in order to identify preclinical at-risk populations eligible for early intervention and intervention trials [6].

In order to improve on research criteria for SCD, The Subjective Cognitive Decline working group (SCD-I) [6] have 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. Cohorts including at-risk participants are recruited by different means, which lead to inclusion of cohorts with different clinical and demographic characteristics. It has been demonstrated that MCI participants recruited through memory clinics harbor more AD-type pathology [14], show a higher prevalence of APOE $\varepsilon$4 alleles [15], are cognitively more impaired [15], and have higher risk of progression to dementia [16, 17] than participants recruited through community or population based samples. Moreover, volunteer sample controls have shown to be better educated and perform better on cognitive tests than population-based samples [15]. However, few studies have investigated the effects of recruitment bias in patients with SCD [18]. Chen et al. [19] recently 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. They attribute this finding to level of concern leading to medical help seeking within the memory clinic sample. Similarly, Perrotin et al. [20] recently published findings demonstrating reduced gray matter volumes and increased depressive symptomatology in SCD cases from a memory clinic sample compared to community-sample. These studies did not demonstrate any differences in cognitive performance at baseline due to recruitment bias. In contrast,

Abdelnour et al. [21] recently 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 pathology [12, 13, 22].

This study compares cognitive performance and demographic characteristics between at-risk participants recruited through memory clinics and participants self-referred by voluntary response to news-bulletins and advertisement. We hypothesize that participants recruited through referrals by a general practitioner (GP) to memory outpatient clinics are more cognitively impaired than participants that are self-referred by voluntary response to news-bulletins or advertisement. We further explore these cohorts by comparing the cognitive symptom groups MCI and SCD to a control group.

## METHODS

This study was a part of "Dementia Disease Initiation" (DDI), a co-operation between all Norwegian health regions and university hospitals. Between January 2013 and January 2017, we recruited participants with self-reported cognitive reduction and healthy controls. For further description of the DDI cohort and methods, see Fladby et al. [23]. All participants were examined following a standard protocol. Participants were recruited from two main sources: 1) self-referred, following advertisements in media, newspapers, or news bulletins, or 2) GP referrals to local memory clinics. Additionally, cognitively healthy controls were included from spouses of patients with dementia/cognitive disorder, and from patients who completed lumbar puncture for orthopedic surgery. Participants were classified as controls, SCD or MCI according to criteria based on a comprehensive assessment program (see below) [6, 24]. The controls were further classified as having either normal or abnormal cognitive screening, and with or without first-degree relative with dementia. We included individuals with a native language of Norwegian, Swedish, or Danish. In order to capture individuals in the preclinical, as well as predementia phases of AD, we included participants between 40 and 80 years of age. 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.

A case report form developed for DDI [23] included assessment protocol for SCD (see below), medical history from participant and informant, physical and neurological examinations, as well as the 15-item Geriatric Depression Score (GDS) [25]. Educational levels were classified in the following categories [26]: 0 = Primary school (7–8 y), 1 = High School (9–11 y), 2 = College (12 y), 3 = Bachelor degree (13–15 y), 4 = Master or equivalent = 16 – 17 y, 5 = Higher university degree/PhD (18–20 y). The cognitive examination included the Mini-Mental State Examination (MMSE-NR) [27], non-verbal cognitive screening (The clock drawing test) [28], verbal memory (CERAD word list) [29], visuoperceptual ability (VOSP silhouettes) [32], psychomotor speed and divided attention (Trail making A and B), and word fluency (COWAT) [30].

The regional medical research ethics committee approved the study. All 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.

### *Classification of SCD and MCI*

The DDI case report form [23] includes a comprehensive account of participants' experience of subjective cognitive decline modelled 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 [6]. MCI was classified according to the NIA-AA criteria which require presence of subjective cognitive decline or impairment combined with lower performance than expected in one or more cognitive domains, yet preserved independence in functional ability and not fulfilling the criteria of dementia [24, 31]. Performance was classified as normal or abnormal according to published norms (adjusted for age, sex, and educational effects) for the different

tests [27–30, 32–34]. Due to overlapping and mutually exclusive criteria, the cut-off values for SCD versus MCI (defined as normal or abnormal cognition) were ≤1.5 standard deviation below normative mean on either CERAD word list (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) [35]. Participants with dementia were excluded if CDR > 0.5 [36].

### *Participants*

Of 577 participants considered, 87 were excluded because they withdrew before finishing the assessment program or did not fulfil the inclusion criteria. Of the 490 participants included, 463 were classified according to disease stage at the time of analysis (Fig. 1). Participants were classified as normal control ($n = 132$, mean age = 60.4, SD = 9.3) or cognitive symptom group ($n = 299$, mean age = 63.7, SD = 9.4), the latter comprising of symptom subgroups MCI ($n = 136$, mean age = 65.4, SD = 9.8) and SCD ($n = 163$, mean age = 62.3, SD = 8.9). Participants who were recruited as normal controls, but had abnormal cognitive screening were excluded from analysis ($n = 32$). Following advertisements in media, we recruited 179 self-referred participants (mean age = 64.4, SD = 9.7), whereas 86 participants (mean age = 61.5, SD = 9.1) were recruited among referrals to local memory clinics. Participants recruited by other means or when recruitment source was not available were excluded from analysis ($n = 34$) [23].

### *Statistical analysis*

For variables with assumed normal distribution (age at inclusion, CERAD word list learning & recall, T-score, VOSP silhouettes T-score, TMT A & B T-score, and COWAT T-score), we compared means for the different groups with one-way ANOVA (analysis of variance) and calculated effect sizes using eta squared ($\eta p^2$). We assessed normality by visual inspection of frequency distributions, Q-Q-plots, and box-plots. Assessing variables with Levene's test, equal variance was assumed for all variables except CERAD word list delayed recall and VOSP silhouettes T-score. Continuous variables with non-normal distribution (MMSE, Clock drawing test) were compared with Mann-Whitney U tests. Education level, being an ordinal variable, was also tested with Mann-Whitney U. We used the Mann-Whitney U z-statistic to calculate effect size $\left(R = \frac{Z}{\sqrt{N}}\right)$ [37]. We compared the binary variable "sex", with Pearson's Chi square test. All analyses were performed in the Statistical Package for Social Sciences (SPSS) version 24.

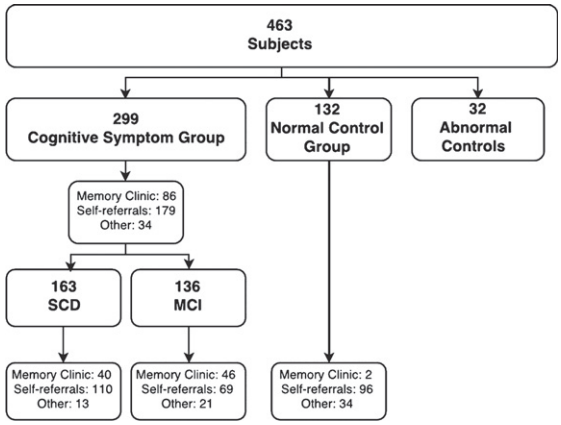


Fig. 1. A total of 463 participants were classified according to disease stage at the time of analysis, whereof 32 recruited as controls showed abnormal cognitive performance and were excluded from analysis. Participants were classified as belonging to a normal control group or cognitive symptom group (SCD and MCI), and their characteristics analyzed depending on recruitment source.

## RESULTS

### *Cognitive performance compared to recruitment strategy*

All data pertaining to comparisons of cognitive performance to recruitment strategies, including demographic characteristics, are shown in Table 1.

No differences in gender distributions were shown between recruitment sources. However, at symptom group level, including both SCD and MCI participants, memory clinic-referrals were shown to be both younger ($p < 0.05$, $\eta p^2 = 0.020$) and less educated ($p < 0.01$, $r = 0.160$) than self-referrals. When measuring the SCD and MCI groups separately, this recruitment bias was only shown for memory clinic-referred MCI participants [age ($p < 0.001$, $\eta p^2 = 0.089$) and education level ($p < 0.05$, $r = 0.201$)]. We found no differences in demographic characteristics between recruitment strategies within the SCD group.

At symptom group level, including both SCD and MCI participants, memory clinic-referrals performed significantly worse than self-referrals on MMSE ($p < 0.05$, $r = 0.138$), Clock drawing test ($p < 0.01$,

Table 1
Demographic characteristics and cognitive test results comparisons between recruitment strategies within the cognitive symptom group (MCI and SCD) and symptom subgroups mild cognitive impairment (MCI) and subjective cognitive decline (SCD)

| | Cognitive symptom Group (MCI & SCD) | | MCI | | SCD | |
|---|---|---|---|---|---|---|
| | Self-referral | Memory clinic referral | Self-referral | Memory clinic referral | Self-referral | Memory clinic referral |
| Age at inclusion | $n = 179$ | $n = 86$ | $n = 69$ | $n = 46$ | $n = 110$ | $n = 40$ |
| *Mean (SD)* | 64.4 (9.7) | 61.5 (9.1) | 67.4 (9.3) | 61.3 (10.3) | 62.5 (9.6) | 61.7 (7.7) |
| | | $\mathbf{p < 0.05}^a$ | | $\mathbf{p < 0.001}^a$ | | $p = \text{n.s.}^a$ |
| | | $\eta\mathbf{p}^2 = \mathbf{0.020}$ | | $\eta\mathbf{p}^2 = \mathbf{0.089}$ | | |
| Female/Total | 96/179 | 45/86 | 33/69 | 21/46 | 63/110 | 24/40 |
| *Percentage female* | 53.6% | 52.3% | 47.8% | 45.7% | 57.3% | 60.0% |
| | | $p = \text{n.s.}^c$ | | $p = \text{n.s.}^c$ | | $p = \text{n.s.}^c$ |
| Education level | $n = 178$ | $n = 86$ | $n = 68$ | $n = 46$ | $n = 110$ | $n = 40$ |
| *Median (IQR)* | 3.0 (2.0) | 2.5 (2.0) | 3.0(2.0) | 2.5 (2.0) | 3.0 (2.0) | 2.5 (2.75) |
| | | $\mathbf{p < 0.01}^b$ | | $\mathbf{p < 0.05}^b$ | | $p = \text{n.s.}^b$ |
| | | $\mathbf{r = 0.160}$ | | $\mathbf{r = 0.201}$ | | |
| MMSE | $n = 178$ | $n = 85$ | $n = 69$ | $n = 45$ | $n = 109$ | $n = 40$ |
| *Mean (SD)* | 28.7 (1.5) | 28.1 (1.9) | 28.0 (1.8) | 27.3 (2.2) | 29.2 (1.1) | 29.1 (1.0) |
| | | $\mathbf{p < 0.05}^b$ | | $p = \text{n.s.}^b$ | | $p = \text{n.s.}^b$ |
| | | $\mathbf{r = 0.138}$ | | | | |
| Clock Drawing Test | $n = 178$ | $n = 84$ | $n = 69$ | $n = 45$ | $n = 109$ | $n = 39$ |
| *Mean (SD)* | 4.9 (0.4) | 4.7 (0.6) | 4.8 (0.5) | 4.6 (0.8) | 4.9 (0.3) | 4.8 (0.4) |
| | | $\mathbf{p < 0.01}^b$ | | $\mathbf{p < 0.05}^b$ | | $p = \text{n.s}^b$ |
| | | $\mathbf{r = 0.188}$ | | $\mathbf{r = 0.186}$ | | |
| CERAD word list Learning T score | $n = 177$ | $n = 84$ | $n = 68$ | $n = 45$ | $n = 109$ | $n = 39$ |
| *Mean (SD)* | 49.7 (12.0) | 43.5 (14.5) | 43.1 (12.7) | 36.0 (12.4) | 53.8 (9.6) | 52.1 (11.7) |
| | | $\mathbf{p < 0.001}^a$ | | $\mathbf{p < 0.01}^a$ | | $p = \text{n.s.}^a$ |
| | | $\eta\mathbf{p}^2 = \mathbf{0.049}$ | | $\eta\mathbf{p}^2 = \mathbf{0.073}$ | | |
| CERAD word list Recall T-score | $n = 176$ | $n = 81$ | $n = 68$ | $n = 41$ | $n = 108$ | $n = 40$ |
| *Mean (SD)* | 47.8 (13.5) | 42.7 (15.1) | 39.5 (14.2) | 34.4 (13.5) | 53.3 (9.9) | 51.2 (11.5) |
| | | $\mathbf{p < 0.01}^a$ | | $p = \text{n.s.}^a$ | | $p = \text{n.s.}^a$ |
| | | $\eta\mathbf{p}^2 = \mathbf{0.030}$ | | | | |
| VOSP Silhouettes T-score | $n = 169$ | $n = 70$ | $n = 65$ | $n = 41$ | $n = 104$ | $n = 29$ |
| *Mean (SD)* | 49.6 (11.2) | 46.5 (11.6) | 44.4 (11.0) | 42.4 (11.2) | 52.9 (10.0) | 52.4 (9.3) |
| | | $p = \text{n.s}^a$ | | $p = \text{n.s.}^a$ | | $p = \text{n.s.}^a$ |
| Trail Making Test A | $n = 177$ | $n = 85$ | $n = 68$ | $n = 45$ | $n = 109$ | $n = 40$ |
| T-score | 45.1 (10.3) | 45.4 (10.3) | 40.4 (10.2) | 41.4 (10.5) | 48.0 (9.4) | 50.0 (8.2) |
| *Mean (SD)* | | $p = \text{n.s.}^a$ | | $p = \text{n.s.}^a$ | | $p = \text{n.s.}^a$ |
| Trail Making Test B | $n = 177$ | $n = 85$ | $n = 68$ | $n = 45$ | $n = 109$ | $n = 40$ |
| T-score | 45.9 (11.1) | 42.5 (12.3) | 40.3 (11.8) | 37.0 (13.0) | 49.5 (9.0) | 48.7 (7.7) |
| *Mean (SD)* | | $\mathbf{p < 0.05}^a$ | | $p = \text{n.s.}^a$ | | $p = \text{n.s.}^a$ |
| | | $\eta\mathbf{p}^2 = \mathbf{0.019}$ | | | | |
| Controlled Oral Word Association Test (COWAT) T-Score | $n = 176$ | $n = 84$ | $n = 68$ | $n = 44$ | $n = 108$ | $n = 40$ |
| Mean (SD) | 49.6 (10.1) | 47.5 (10.7) | 46.6 (10.0) | 44.2 (10.6) | 51.5 (9.7) | 51.0 (9.7) |
| | | $p = \text{n.s.}^a$ | | $p = \text{n.s.}^a$ | | $p = \text{n.s.}^a$ |

The continuous variables *(Age at inclusion, CERAD word list learning and recall T-score, VOSP silhouettes T-score, TMT-A and TMT-B T-score and COWAT T-score)* are summarized by mean (standard deviation, SD). The ordinal variable *educational level* is described by median (interquartile range). Variables of assumed normal distribution are compared with one-way ANOVA with predefined contrasts and effect sizes ($\eta p^2$) are provided for significant results (a). Variables of non-normal distribution (*MMSE* and *Clock drawing test*) and the ordinal variable (*education level*) are compared with Mann-Whitney U tests and effect sizes (r) are provided for significant results (b). The binary variable *sex* is described with observed numbers and percentages and compared with Pearson's Chi square tests (c). Significant *p*-values and effect sizes are shown in Bold.

$r = 0.188$), CERAD word list learning ($p < 0.001$, $\eta p^2 = 0.049$), CERAD word list recall ($p < 0.01$, $\eta p^2 = 0.030$), and trail making test B ($p < 0.05$, $\eta p^2 = 0.019$). However, within the MCI group, this performance deficit was only shown for the clock drawing test ($p < 0.05$, $r = 0.186$) and CERAD word list learning ($p < 0.01$, $\eta p^2 = 0.073$). Within the SCD group we found no significant differences in cognitive performance between self-referrals and memory clinic referrals.

Table 2
Demographic characteristics and cognitive test result comparisons between control group and cognitive symptom group (SCD and MCI), as well as subgroups with mild cognitive impairment (MCI) and subjective cognitive decline (SCD)

| | Control Group | Cognitive symptom Group (MCI & SCD) | MCI | SCD |
|---|---|---|---|---|
| Age at inclusion | $n = 132$ | $n = 299$ | $n = 136$ | $n = 163$ |
| *Mean (SD)* | 60.4 (9.3) | 63.7 (9.4) | 65.4 (9.8) | 62.3 (8.9) |
| | | $\mathbf{p < 0.001}^a$ | $\mathbf{p < 0.001}^a$ | $p = \text{n.s.}^a$ |
| | | $\eta\mathbf{p}^2 = \mathbf{0.026}$ | $\eta\mathbf{p}^2 = \mathbf{0.064}$ | |
| Female/Total | 75/132 | 155/299 | 63/136 | 92/163 |
| Percentage female | 56.8% | 51.8% | 46.3% | 56.4% |
| | | $p = \text{n.s.}^c$ | $p = \text{n.s.}^c$ | $p = \text{n.s.}^c$ |
| Education level | $n = 131$ | $n = 298$ | $n = 135$ | $n = 163$ |
| Median (IQR) | 3.0 (2.0) | 3.0 (2.0) | 3.0 (3.0) | 3.0(3.0) |
| | | $p = \text{n.s.}^b$ | $p = \text{n.s.}^b$ | $p = \text{n.s.}^b$ |
| MMSE | $n = 131$ | $n = 296$ | $n = 134$ | $n = 162$ |
| Mean (SD) | 29.4 (0.9) | 28.4 (1.7) | 27.6 (2.0) | 29.1 (1.1) |
| | | $\mathbf{p < 0.001}^b$ | $\mathbf{p < 0.001}^b$ | $\mathbf{p < 0.05}^b$ |
| | | $\mathbf{r = 0.278}$ | $\mathbf{r = 0.514}$ | $\mathbf{r = 0.126}$ |
| Clock Drawing Test | $n = 130$ | $n = 293$ | $n = 134$ | $n = 159$ |
| Mean (SD) | 4.9 (0.3) | 4.8 (0.5) | 4.7 (0.6) | 4.9 (0.3) |
| | | $\mathbf{p < 0.01}^b$ | $\mathbf{p < 0.001}^b$ | $p = \text{n.s.}^b$ |
| | | $\mathbf{r = 0.130}$ | $\mathbf{r = 0.250}$ | |
| CERAD word list Learning T score | $n = 130$ | $n = 293$ | $n = 133$ | $n = 160$ |
| *Mean (SD)* | 56.1 (9.5) | 47.8 (13.0) | 40.9 (12.8) | 53.5 (10.2) |
| | | $\mathbf{p < 0.001}^a$ | $\mathbf{p < 0.001}^a$ | $\mathbf{p < 0.05}^a$ |
| | | $\eta\mathbf{p}^2 = \mathbf{0.092}$ | $\eta\mathbf{p}^2 = \mathbf{0.313}$ | $\eta\mathbf{p}^2 = \mathbf{0.016}$ |
| CERAD word list Recall T-score | $n = 130$ | $n = 289$ | $n = 130$ | $n = 160$ |
| *Mean (SD)* | 55.2 (11.2) | 46.1 (14.5) | 37.5 (14.4) | 53.0 (10.3) |
| | | $\mathbf{p < 0.001}^a$ | $\mathbf{p < 0.001}^a$ | $p = \text{n.s.}^a$ |
| | | $\eta\mathbf{p}^2 = \mathbf{0.087}$ | $\eta\mathbf{p}^2 = \mathbf{0.320}$ | |
| VOSP Silhouettes T-score | $n = 120$ | $n = 268$ | $n = 126$ | $n = 142$ |
| *Mean (SD)* | 52.7 (8.9) | 48.2 (11.2) | 43.6 (10.9) | 52.3 (9.8) |
| | | $\mathbf{p < 0.001}^a$ | $\mathbf{p < 0.001}^a$ | $p = \text{n.s}^a$ |
| | | $\eta\mathbf{p}^2 = \mathbf{0.038}$ | $\eta\mathbf{p}^2 = \mathbf{0.175}$ | |
| Trail Making Test A | $n = 128$ | $n = 293$ | $n = 132$ | $n = 161$ |
| T-score | 49.2 (9.6) | 45.0(10.2) | 40.7 (9.9) | 48.6 (8.9) |
| *Mean (SD)* | | $\mathbf{p < 0.001}^a$ | $\mathbf{p < 0.001}^a$ | $p = \text{n.s.}^a$ |
| | | $\eta\mathbf{p}^2 = \mathbf{0.035}$ | $\eta\mathbf{p}^2 = \mathbf{0.157}$ | |
| Trail Making Test B | $n = 127$ | $n = 293$ | $n = 132$ | $n = 161$ |
| T-score | 51.6 (8.8) | 44.9 (11.7) | 39.5 (12.4) | 49.4 (9.0) |
| *Mean (SD)* | | $\mathbf{p < 0.001}^a$ | $\mathbf{p < 0.001}^a$ | $\mathbf{p < 0.05}^a$ |
| | | $\eta\mathbf{p}^2 = \mathbf{0.074}$ | $\eta\mathbf{p}^2 = \mathbf{0.243}$ | $\eta\mathbf{p}^2 = \mathbf{0.015}$ |
| Controlled Oral Word | $n = 128$ | $n = 290$ | $n = 131$ | $n = 159$ |
| Association Test | 50.5 (7.6) | 48.8 (10.4) | 45.5 (10.1) | 51.6 (9.8) |
| (COWAT) T-Score | | $p = \text{n.s.}^a$ | $\mathbf{p < 0.001}^a$ | $p = \text{n.s.}^a$ |
| *Mean (SD)* | | | $\eta\mathbf{p}^2 = \mathbf{0.072}$ | |

The continuous variables *(Age at inclusion, CERAD word list learning and recall T-score, VOSP silhouettes T-score, TMT-A and TMT-B T-score and COWAT T-score)* are summarized by mean (standard deviation, SD). The ordinal variable *educational level* is described by median (interquartile range). Variables of assumed normal distribution are compared with one-way ANOVA with predefined contrasts and effect sizes ($\eta p^2$) are provided for significant results (a). Variables of non-normal distribution (*MMSE* and *Clock drawing test*) and the ordinal variable (*education level*) are compared with Mann-Whitney U tests and effect sizes (r) are provided for significant results (b). The binary variable *sex* is described with observed numbers and percentages and compared with Pearson's Chi square tests (c). Significant *p*-values and effect sizes are shown in Bold.

### *Cognitive symptom groups compared to control group*

All data pertaining to control and symptom group comparisons including demographic characteristics are shown in Table 2.

There were no differences in educational level or gender distributions between groups. However, the symptom group was significantly older than the control group ($p < 0.001$, $\eta p^2 = 0.026$). This difference was attenuated when comparing the MCI group to controls ($p < 0.001$, $\eta p^2 = 0.064$), but not

significant when comparing the SCD group to controls. Moreover, a further analysis of the control group characteristics showed that a majority was recruited through the spouses of participants responding to advertisements ($n = 96$, 72.7%), whereas 25.8% ($n = 34$) were recruited by other means, and only 1.5% ($n = 2$) were recruited through the spouses of memory clinic-referrals.

At symptom group level, including both MCI and SCD participants, we found significantly reduced test performances compared to controls on all measures ($p < 0.01$), except the controlled oral word association test (COWAT). When comparing controls to MCI participants, significant reductions were found for all measures, including COWAT ($p < 0.001$, see Table 2 for details). SCD participants performed significantly worse compared to controls on MMSE ($p < 0.05$, $r = 0.126$), CERAD word list learning ($p < 0.05$, $\eta p^2 = 0.016$), and trail making test B ($p < 0.05$, $\eta p^2 = 0.015$).

## DISCUSSION

In agreement with our hypothesis, memory clinic-referrals performed worse on cognitive tests than self-referred individuals. In addition, they were both younger and less educated compared to self-referrals. However, when comparing the MCI and SCD groups separately, only the MCI group showed these recruitment biases. We found no differences in cognitive performance or demographic characteristics between recruitment strategies within the SCD group. Further, we found that the SCD group performed worse on both cognitive screening (MMSE), and the cognitive subtests; word list learning (CERAD), and divided attention (TMT-B) compared to an age and education matched control group. In addition, the MCI group was shown to be older compared to controls, but did not differ in gender distributions or educational level.

In line with earlier reports, our findings show that including at-risk patients from memory clinics preferentially recruit individuals who are more cognitively impaired compared to self-referred individuals from the community. These findings generally support the notion that inclusion from memory clinics recruit individuals that are at higher risk of conversion to dementia [16, 17] or are farther along the disease trajectory than participants recruited through other means [14, 15]. Moreover, the MCI participants recruited through memory clinics, while more cognitively impaired, were also younger and might represent an earlier onset, or more aggressive form of pathology than found in the older self-referred sample. Memory clinic samples have shown to harbor higher risk in terms of genetic risk factors [15], higher presence of AD-type pathology [38], or more aggressive forms of pathology [14]. 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 [39], thus lower cognitive performance in this group may be confounded with a lesser ability to compensate for brain pathology compared to the self-referred group. Lastly, our control group comprised nearly 73% advertisement recruited individuals. It has been shown that controls recruited from convenience samples tend to be younger, better educated, and perform better on cognitive tests than controls recruited through population samples [15]. As such, our control group may not be an adequate comparison to memory clinic-referrals. However, although the cognitive symptom group was found to be older than controls, no difference was found in educational levels. Moreover, apart from the clock drawing test and MMSE, cognitive test scores were adjusted for effects of age and educational levels making between group comparisons possible.

We found no significant differences in either demographics or cognitive performance due to recruitment bias within the SCD group. This is perhaps not surprising given the fact that a core criterion for SCD is normal range of scores on neuropsychological examinations [6]. Any subtle differences between recruitment methods may be too small for detection within this group. Furthermore, recruitment did not bias other key demographics, leaving self- and memory clinic-referrals matched on these variables. To our knowledge, only one study to date have shown recruitment source to preferentially bias cognitive performance in SCD cases [21]. However, there are key differences in sample characteristics between studies. While both studies recruited participants from memory clinics, Abdelnour et al. [21] recruited participants from an open house initiative (OHI). This initiative offered free examinations to the community and did not specifically recruit participants to a memory study. In addition, the OHI SCD cases were more likely to be female and had higher educational levels compared to the memory unit sample. Conversely, regardless of recruitment source, participants within the DDI cohort were recruited specifically for a study on cognitive reduction. Moreover, we found no significant recruitment bias in demographics within the

SCD group. Thus, regardless of recruitment source, the DDI SCD participants may be more similar within the DDI cohort, and thereby showing similar levels of cognitive performances. However, although not reaching the level of statistical significance, the data did show 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 ($n = 40$)), and could have reached statistical significance given a larger sample. Moreover, we did find an overall significant difference in cognitive performance at symptom group level 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.

While recruitment source did not significantly bias cognitive performance or demographics, we observed a relative increase in depressive symptoms measured by the GDS 15 in the memory clinic-referred SCD cases compared to self-referrals (data not shown). However, the observed increase in symptoms was not above the suggested cut-offs for clinical depression at group level [40]. 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. As such, 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 recent study by Perrotin et al. [20] comparing SCD cases recruited from memory clinics and community sample, showed significant reductions in gray matter volumes related to AD pathology in the memory clinic group. The authors conclude that medical help seeking and increased depressive symptoms were related to these volume reductions and pointing out increased affective burden as a potential part of prodromal AD. Conversely, Heser et al. [41] 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. As such, depressive symptoms even at subclinical levels may be an important factor in preclinical AD. This should be further explored in future studies focusing on the trajectory of preclinical AD development.

While not demonstrating statistically significant recruitment bias in cognitive performance, the present study shows that the SCD group 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 [6]. A recent review by Garcia-Ptacek et al. [42] summarizes that, although most studies show poorer cognitive performance in persons with subjective cognitive complaints, such findings have not been universally supported. Furthermore, reductions within these domains may be influenced by other factors such as personality, anxiety or depressive symptoms, or accounted for by other medical or neurological disorders other than AD. Both CERAD word list learning and Trail making test B rely on adequate working memory and attentional processes, both of which can be affected by numerous conditions. Also, the SCD group did not perform worse than controls on memory recall. These findings are therefore not unequivocally linked to AD-pathology and may represent different conditions or disease etiologies. Moreover, the control and SCD group performed very similar in most of the cognitive measures, which may suggest that AD enrichment within the SCD group is relatively low. Future studies combining biological markers with SCD phenotypes and follow-ups are needed to ascertain the meaning of this finding.

The present study has some limitations that 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. Second, our study is limited to a cross sectional comparison of cohort characteristics and does not include outcome measure of disease progression. Third, we did not include the use of biomarker evidence to further characterize selection bias, limiting interpretation of current findings. Fourth, the inclusion criteria allow the recruitment of younger middle aged adults (40–80 y), which lowers the mean age and increases variability in our sample. Advancing age is a well-known risk factor for AD. Thus, while this is an optimal design to capture early preclinical disease events in a longitudinal study, it may lead to dilution of AD prevalence in

both SCD and MCI samples in our cross-sectional analysis. Lastly, an important note has to be made on the use of Sotaniemi et al. [34] CERAD word list normative dataset. 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 lead to uncertain classification of MCI and SCD. As such, there may be a need to establish normative datasets better suited for younger pre-clinical at-risk cohorts.

A central aim of the DDI project is to examine incipient disease activity to detect and track dementia disease progression in its preclinical states. Therefore, the cohort is comprised of a younger sample than most previous large cohort studies and the characteristics of this sample with regard to the impact of biomarker findings and longitudinal outcomes are not yet known. Future studies on the DDI cohort employing longitudinal designs and utilizing both biological and psychological data will serve to further delineate the clinical significance of these findings to define the characteristics of SCD due to AD more closely.

### *Conclusions*

Our findings indicate that recruitment through memory clinics preferentially includes participants at higher risk of dementia, or are more advanced than cases recruited through other means. In addition, SCD cases were shown to perform worse on key cognitive measures compared to controls and may suggest that SCD is a symptom of subtle cognitive decline. Recruitment was not shown to significantly bias demographic characteristics or cognitive performance within the SCD group alone. However, at symptom group level, we did find an overall significant effect of worse cognitive performance in memory clinic-referrals beyond what was shown by the MCI group alone. This suggests that although the differences in cognitive performance are small, SCD cases recruited from memory clinics may represent a cognitively more impaired group than self-referred SCD cases. These findings suggest that recruitment source affects clinical characteristics of preclinical cohorts and should be taken into consideration when comparing findings between studies utilizing different recruitment methods. Future studies employing longitudinal designs and combining psychological and biological data are needed to further delineate the significance of these findings, as well as addressing the impact of recruitment bias on biological risk-factors within the DDI cohort.

## ACKNOWLEDGMENTS

The project was funded by Norwegian Research Council, NASATS (Dementia Disease Initiation) and the JPND (APGeM) and funding from the regional health authorities (Helse Sør-Øst and Helse Nord). We thank Svein Ivar Bekkelund, Kjell-Arne Arntzen, Kai Müller, Torgil Riise Vangberg, Claus Albretsen, Elisabeth Gundersen, Mari Thoresen Løkholm, Ida Harviken, Line Sæther, Erna Utnes, Marianne Wettergreen, Berglind Gisladottir, Marit Knapstad, Reidun Meling, and Synnøve Bremer Skarpenes for clinical examinations and essential help with the project.

Authors' disclosures available online (http://j-alz.com/manuscript-disclosures/17-0385r1).

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Alzheimer's & Dementia: Translational Research & Clinical Interventions 4 (2018) 617-627

Featured Article

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

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**Abstract**

**Introduction:** The cerebrospinal fluid neurogranin (Ng)/β-site amyloid precursor protein-cleaving enzyme 1 (BACE1) ratio may reflect synaptic affection resulting from reduced beta-amyloid (Aβ) clearance. We hypothesize that increased Ng/BACE1 ratio predicts the earliest cognitive decline in Alzheimer's disease.

**Methods:** We compared Ng/BACE1 levels between cases with subjective cognitive decline (n = 18) and mild cognitive impairment (n = 20) both with amyloid plaques and healthy controls (*APOE*-ε4+, n = 16; *APOE*-ε4-, n = 20). We performed regression analyses 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:** Ng/BACE1 levels were elevated in both subjective cognitive decline and mild cognitive impairment compared to healthy controls. Higher Ng/BACE1 ratio was associated with lower hippocampal and amygdala volumes; lower baseline memory functions, attention, and MMSE; and significant decline in MMSE and memory function at 2-year follow-up.

**Discussion:** High Ng/BACE1 ratio predicts cognitive decline also in preclinical cases with amyloid plaques.



*Keywords:* Alzheimer's disease; MCI (mild cognitive impairment); SCD (subjective cognitive decline); MRI; Memory; Cognition; Synaptic loss; Cerebrospinal fluid (CSF); CSF neurogranin; CSF BACE1

Declarations of Interest: K.N. receives publishing royalties from the popular science books "Hjernen er stjernen" from 2016 and "Hjernetrening" from 2018 published at Kagge Forlag AS (Oslo, Norway) and gets honoraria for talks with the same title from Athenas speakers' bureau (Tønsberg, Norway). B.B. is a full-time employee of EUROIMMUN AG. E.V. is the co-founder of ADx NeuroSciences, Zwijnaarde, Belgium, and founder of Key4AD, Eke, Belgium. D.A. has received research support and/or honoraria from Astra-Zeneca, H. Lundbeck, Novartis Pharmaceuticals, and GE Health, and serves as paid consultant for H. Lundbeck, Eisai, and Axovant. D.A. is a Royal Society Wolfson Research Merit Award Holder and would like to thank the Wolfson Foundation and the Royal Society for their support. Other authors have no conflicts of interest to disclose.

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https://doi.org/10.1016/j.trci.2018.10.003

## 1. Introduction

In Alzheimer's disease (AD), amyloid-β precursor protein (AβPP) metabolizes to Aβ-peptide, which precipitates in amyloid plaques [1]. Increased CSF neurogranin is related to synaptic loss, cognitive decline, and reductions in hippocampal volume in mild cognitive impairment (MCI) and dementia due to AD. Moreover, increased CSF neurogranin may distinguish AD from other neurodegenerative diseases [2–5]. Previously, we showed an inverse relationship between CSF neurogranin and the CSF $A\beta_{1–42}/A\beta_{1–40}$ ratio in MCI and dementia, suggesting that synaptic loss and AβPP metabolism may be linked [6]. Neurogranin is highly expressed in dendritic spines in hippocampal and amygdalar pyramidal cells and is linked to postsynaptic signal transduction [7,8]. The β-site amyloid precursor protein-cleaving enzyme 1 (BACE1) is linked to presynaptic AβPP metabolism [9,10]. Aβ-oligomers accumulate at synaptic terminals and may disrupt pyramidal cell N-methyl-D-aspartate (NMDA) receptors and postsynaptic $Ca^{2+}$ homeostasis [11–13], putatively leading to synapse loss. The *APOE*-ε4 allele is a major genetic risk factor for AD and may enhance synaptotoxic oligomerization of Aβ-peptides [11,14,15].

As BACE1 is a rate-limiting step in the production of Aβ species [9,10], inhibitors are tested [16]. Clinical and biomarker studies in AD cases have shown contradictory results [17,18]. CSF $A\beta_{1–42}$, as a marker for amyloid plaques (A), and CSF phosphorylated and CSF total tau, as markers for neurofibrillary tangles (T) and neurodegeneration (N), have been combined to the A/T/N stage marker for AD [19]. BACE1 levels have been shown to correlate with markers of neuronal degradation and neurofibrillary tangles (total and phosphorylated tau) [20], as well as synaptic loss (neurogranin), but not with Aβ [21], suggesting a relationship to neurodegeneration. Associated biomarkers can be explored as ratios, which, in some cases, have shown to offer better diagnostic performance, for example, the CSF $A\beta_{1–42}/A\beta_{1–40}$ ratio [22]. Recently, we compared several CSF measures as single analytes and ratios to cognitive decline and found that an increased ratio between CSF neurogranin trunc P75 and BACE1 (Ng/BACE1) was the only robust correlate of cognitive decline in MCI cases due to AD [21]. We propose that this ratio could sensitively reflect early synapse affection in AD linked to accumulation of toxic Aβ-oligomers at synaptic terminals.

Thus, we hypothesize that increased Ng/BACE1 ratio may herald development of cognitive deficits at a preclinical stage of AD [23,24]. To test this hypothesis, we included cases early in the AD trajectory (i.e., cases with subjective cognitive decline (SCD) and MCI with amyloid plaques) [19,25] and healthy *APOE*-ε4+ and *APOE*-ε4- control groups. We compared levels of Ng/BACE1 between the groups, relate Ng/BACE1 to AD biomarker severity using the A/T/N classification scheme [19], and explore relationships to baseline hippocampal and amygdala volumes and cognitive decline at 2-year follow-up.

## 2. Methods and materials

### 2.1. The Dementia Disease Initiation cohort

This study was a part of the Norwegian multicenter study, "Dementia Disease Initiation" (DDI) [26]. DDI uses a standardized protocol for participant selection, assessment, and disease-stage classification (SCD, MCI, and dementia) according to published criteria [25,27,28]. Participants were recruited from referrals to local memory clinics or self-referrals responding to advertisements in media, newspapers, or news bulletins. Healthy controls were recruited from spouses of participants with either MCI or SCD, volunteers responding to media advertisements or news bulletins, and from cognitively healthy patients who completed lumbar puncture for orthopedic surgery. Criteria for inclusion were age between 40 and 80 years and a native language of Norwegian, Swedish, or Danish. Exclusion criteria were brain trauma or disorder, including clinical stroke, dementia, severe psychiatric disorder, severe somatic disease that might influence the cognitive functions, intellectual disability, or other developmental disorders. The cohort described here was recruited between 2013 and 2017. For further description of the DDI cohort and methods, refer to the study by Fladby et al. (2017) [26]. Participants were assessed at baseline, and a subset had come to 2-year follow-up examination.

### 2.2. CSF collection and handling

Procedures were as described previously [26]. All CSF samples were analyzed at the Department of Interdisciplinary Laboratory Medicine and Medical Biochemistry at Akershus University Hospital, and samples from all other sites were frozen before sending to this laboratory following BIOMARKAPD SOPs as also described previously [29].

### 2.3. Protein biomarker measurements

Commercial enzyme-linked immunosorbent assays based on monoclonal antibodies were used to measure CSF levels of the following protein biomarkers: $A\beta_{1–42}$, t-tau, and p-tau were determined using Innotest Aβ (1–42), Innotest h-Tau Ag, and Innotest Phospho-Tau (181P) (Fujirebio, Ghent, Belgium), respectively. BACE1 and neurogranin (trunc P75) levels were determined using kits from EUROIMMUN AG (Lübeck, Germany) as described in detail elsewhere [21]. 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.

### 2.4. Participant selection, study design, and A/T/N classification

For the purposes of the present study, we selected participants from the DDI cohort to construct four groups according to the 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; and (4) MCI (n = 20) with CSF confirmed amyloid pathology. In addition, participants were classified according to the A/T/N classification scheme for AD using CSF biomarkers [19]. **A**+ denotes (CSF amyloid pathology only), **A** + **N**+ (CSF amyloid pathology and neurodegenerative marker), and **A** + **N** + **T**+ (CSF amyloid pathology, neurodegenerative marker, and marker of neurofibrillary tangles). The following cutoff values for CSF total tau (t-tau) and phosphorylated tau (p-tau) abnormality were applied according to the laboratory recommendations (modified from the study by Sjögren et al. [30]); t-tau is >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. An optimal cutoff at CSF $A\beta_{1–42} < 708$ for amyloid plaque pathology was determined following DDI PET [$^{18}$F]-flutemetamol uptake studies [31]. Amyloid-positive cases were screened in accordance with the A/T/N classification scheme [19] before inclusion to ensure equal distribution of pathological markers between SCD and MCI groups. For demographics and study cohort characteristics, please see Table 1.

### 2.5. Neuropsychological battery

The neuropsychological battery included the Mini Mental State Examination (MMSE-NR) [32], verbal learning and memory recall (CERAD word list test) [33], psychomotor speed, and divided attention (trail-making test A and B [TMT A and B]). T-scores for the trail-making tests were calculated using published norms [34]. For the CERAD word list test, we used the normative performance of the DDI cohort control group [26] to calculate T-scores after a recent article that showed published norms not matching the younger and more educated DDI cohort [35]. A total of 42 of 74 baseline cases had available cognitive data at 2-year follow-up.

### 2.6. Magnetic resonance imaging

Magnetic resonance imaging (MRI) was performed at 7 sites, and 7 scanners were used; a total of 57 MRI scans 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 using

Table 1
Between-group comparisons between demographics, cognitive, AD, and A/T/N biomarker characteristics and *APOE*-ε4+/− distribution

| | Groups | | | | | ANOVA contrasts (*P*)/Dunn's pairwise comparisons | | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | *APOE*-ε4− controls (n = 20) | *APOE*-ε4+ controls (n = 16) | Aβ+ SCD (n = 18) | Aβ+ MCI (n = 20) | $F/\chi^2$ and $\eta p^2/\eta^2$ (*P*) | 1 vs 2 | 3 vs 1 and 2 | 4 vs 1 and 2 | 3 vs 4 |
| Age mean (SD) | 62.8 (9.6) | 59.1 (8.5) | 66.7 (6.8) | 66.8 (7.4) | $F = 4.3$, $\eta p^2 = .14$ (<.01) | *n.s.* | <.05 | <.01 | *n.s* |
| Female, n (%) | 10 (50%) | 9 (56%) | 8 (44%) | 12 (57%) | $\chi^2 = 0.8$, $\eta^2 = .23$ (n.s.) | * | * | * | * |
| MMSE mean (SD) | 29.4 (0.7) | 29.5 (0.7) | 29.2 (0.8) | 26.9 (2.2) | $\chi^2 = 19.4$ (<.0001) | *n.s.* | *n.s.* | <.001 | <.01 |
| CERAD learning T-score mean (SD) | 47.8 (10.8) | 54.1 (10.7) | 49.6 (8.2) | 36.3 (10.3) | $F = 10.1$, $\eta p^2 = .31$(<.001) | *n.s.* | *n.s.* | <.001 | <.001 |
| CERAD recall T-score mean (SD) | 45.1 (13.3) | 55.0 (6.1) | 50.4 (10.0) | 35.1 (10.5) | $\chi^2 = 25.2$, $\eta^2 = .32$ (<.001) | *n.s.* | *n.s.* | <.001 | <.001 |
| TMT-A T-score mean (SD) | 50.2 (10.5) | 49.3 (7.8) | 50.3 (6.4) | 41.0 (6.7) | $F = 6.2$, $\eta p^2 = .22$ (<.001) | *n.s.* | *n.s.* | <.001 | <.01 |
| TMT-B T-score mean (SD) | 54.2 (7.2) | 52.0 (9.5) | 48.7 (7.9) | 39.5 (9.7) | $F = 10.3$, $\eta p^2 = .32$ (<.001) | *n.s.* | *n.s.* | <.001 | <.05 |
| CSF $A\beta_{1–42}$ mean (SD) | 1082 (188) | 996 (175) | 530 (98) | 496 (117) | $\chi^2 = 56.2$, $\eta^2 = .76$ (<.001) | *n.s.* | <.0001 | <.0001 | *n.s.* |
| CSF t-tau mean (SD) | 302 (99) | 293 (97) | 487 (249) | 543 (284) | $\chi^2 = 15.9$, $\eta^2 = .18$ (<.001) | *n.s.* | <.05 | <.05 | *n.s.* |
| CSF p-tau mean (SD) | 50 (12) | 52 (14) | 74 (33) | 82 (44) | $\chi^2 = 12.6$, $\eta^2 = .14$ (<.0001) | *n.s.* | <.05 | <.05 | *n.s.* |
| A + T−N− n (%) | | | 9 (50%) | 11 (52%) | † | † | † | † | † |
| A + T−N+ n (%) | | | 2 (11%) | 2 (10%) | † | † | † | † | † |
| A + T+N+ n (%) | | | 7 (39%) | 8 (38%) | † | † | † | † | † |
| *APOE*-ε4 n (%) | 0 (0 %) | 16 (100%) | 13 (72%) | 15 (74%) | † | † | † | † | † |

Abbreviations: n.s., nonsignificant result; Aβ+, CSF confirmed amyloid pathology; *APOE*-ε4+/−, apolipoprotein E 4 allele positive or negative; SCD, subjective cognitive decline; MCI, mild cognitive impairment; SD, standard deviation; ANOVA, analysis of variance; MMSE, Mini Mental State Examination; TMT, trail-making test; AD, Alzheimer's disease; CSF, cerebrospinal fluid.

*No contrasts/post hoc tests performed.

†No statistical tests applied.

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 using 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 using 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 using 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 using a GE Optima Medical Systems 1.5 Tesla system (GE Healthcare, Chicago, IL). 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) was obtained. Finally, 1 MRI scan was performed using 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.

### 2.7. 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 [36]. 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.

### 2.8. Statistical analysis

Normality was assessed through the inspection of QQ-plots, histograms, and the Shapiro-Wilk test of normality.

To assess differences in biomarker levels, MRI-derived medial temporal lobe (MTL) volumes, cognitive tests, and demographics between groups, we performed one-way analyses of variance (ANOVAs) with planned comparisons for variables with normal distributions. For MTL volumes, ANOVA analyses were performed on standardized residuals after covariate regression correction for age, gender, and MRI scanner model. We performed Kruskal-Wallis test with Dunn's nonparametric pairwise post hoc test to assess group differences in variables with non-normal distributions (CSF $A\beta_{1-42}$, CSF t-tau, CSF t-tau, CERAD recall T-score, and MMSE). Nonparametric pairwise comparisons and ANOVA contrasts were performed in a hierarchical manner. If the high- and low-risk control groups were found equal on the relevant measure, we proceeded to compare SCD and MCI groups to controls (collapsed control group) and finally comparing the SCD with the MCI group. The dichotomous variable "gender" was assessed using a chi-square test. To compare levels of CSF neurogranin, CSF BACE1, and their ratio score to groups derived from the A/T/N groups, one-way ANOVAs with post hoc Bonferroni corrections were performed. Effect sizes are provided for ANOVA ($\eta p^2$) and Kruskal-Wallis test ($\eta^2$) [37].

The impact of CSF biomarkers on MMSE scores were assessed using a multiple linear regression model controlling for age, and simple linear regression models were fitted to assess the relationship between biomarkers and age-adjusted T-scores for the different cognitive tests at baseline. Similarly, the relationships between biomarkers and MTL volumes were assessed using several multiple regression analyses controlling for effects of age, gender, and MRI scanner variant. Effect sizes for the overall regression models are provided ($R^2$).

Because CSF $A\beta_{1-42}$ was used as core selection criteria in the study design, it was omitted as predictor from baseline regression analyses with cognitive and MRI variables. However, we assessed CSF $A\beta_{1-42}$ as the predictor of cognitive changes at 2-year follow-up. CSF p-tau and t-tau demonstrated collinearity (variance inflation factor $> 7$). Thus, only CSF total tau was included in our regression models.

To assess the 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.

All analyses were performed in the Statistical Package for Social Sciences (SPSS) version 24.

### 2.9. 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. Results

### 3.1. Between-group CSF biomarker comparisons

We found significantly increased levels of CSF Ng/BACE1 in both SCD ($t(71) = 2.532$, $P < .05$) and MCI ($t(71) = 3.595$, $P < .001$) compared with controls.

Table 2
Between-group comparisons between CSF biomarkers and MTL volumetry

| Variable | Groups | | | | | ANOVA contrasts (*P*) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *APOE*-ε4− controls (n = 20) | *APOE*-ε4+ controls (n = 16) | Aβ+ SCD (n = 18) | Aβ+ MCI (n = 20) | *F* and $\eta p^2$ (*P*) | 1 vs 2 | 3 vs 1 and 2 | 4 vs 1 and 2 | 3 vs 4 |
| CSF Ng mean (SD) | 390 (143) | 355 (108) | 468 (217) | 428 (179) | $F = 1.5$ (n.s) | * | * | * | * |
| CSF BACE1 mean (SD) | 2289 (547) | 2140 (374) | 2442 (1132) | 2064 (679) | $F = 0.4$ (n.s) | * | * | * | * |
| CSF Ng/BACE1 mean (SD) | .1659 (.03) | .1635 (.03) | .1921 (.04) | .2022 (.05) | $F = 4.9$, $\eta p^2 = .17$ (<.01) | *n.s.* | <.05 | <.01 | *n.s.* |
| Hippocampus average volume mean (SD) | 23.0 (2.9) | 22.4 (3.9) | 21.4 (3.3) | 19.4 (3.8) | $F = 2.3$† (n.s.) | * | * | * | * |
| Amygdala average volume mean (SD) | 1.2 (0.3) | 1.1 (0.2) | 1.0 (0.2) | 0.9 (0.2) | $F = 1.8$† (n.s.) | * | * | * | * |

Abbreviations: n.s., nonsignificant result; Aβ+, CSF confirmed amyloid pathology; *APOE*-ε4+/−, apolipoprotein E 4 allele positive or negative; CSF, cerebrospinal fluid; MTL, medial temporal lobe; ANOVA, analysis of variance; Ng, neurogranin; SD, standard deviation; BACE1, β-site amyloid precursor protein-cleaving enzyme 1.

*Contrasts or post hoc tests not performed due to non-significant ANOVA.

†Between-group comparisons of MRI medial temporal volumetry are performed on standardized residuals following covariate regression correction for age, gender and MRI scanner variant.

No differences were demonstrated between SCD and MCI groups or even between the high- vs. low-risk control groups (Table 2 and Fig. 1). Moreover, no significant between-group differences were found for Ng or for BACE1 when measured separately (Table 2).

### 3.2. CSF biomarkers in relation to A/T/N groups

Both CSF Ng ($F(3,69) = 8.801$, $\eta p^2 = .28$, $P < .0001$) and CSF BACE1 ($F(3,69) = 7.201$, $\eta p^2 = .24$, $P < .0001$), as well as CSF Ng/BACE1 ratio ($F(3,69) = 6.656$, $\eta p^2 = .22$, $P < .0001$), were significantly different between A/T/N groups.

Levels of CSF Ng/BACE1 were increased in the **A**+ **N**+ group (n = 10, $M = .2102$, standard deviation [SD] = .05) compared with controls (n = 35, $M = .1642$, $SD = .03$, $P < .01$). However, this was not shown for Ng or BACE1 when measured separately. Both CSF BACE1 (n = 13, $M = 2884$, $SD = 958$, $P < .05$) and Ng levels ($M = 580$, $SD = 164$, $P < .0001$), as well as Ng/BACE1 level ($M = .2061$, $SD = .04$, $P < .01$), were elevated in the **A** + **T**+**N**+ group compared with individuals with normal CSF (Ng: $M = 369$, $SD = 126$; Ng/BACE1: $M = .1642$, $SD = .03$). In addition, Ng (n = 13, $M = 580$, $SD = 164$) was also elevated in the **A** + **T**+**N**+ group compared with the **A**+ group (n = 15, $M = 323$, $SD = 129$, $P < .0001$). No significant differences between healthy controls with normal CSF and amyloid-positive (**A**+) individuals were found for CSF BACE1, Ng, or Ng/BACE1.

### 3.3. CSF biomarkers, APOE-ε4, and MRI-derived medial temporal volumetry

All models include covariates controlling for age, gender, and scanner variant. When analyzing the entire sample (n = 57), higher CSF Ng/BACE1 levels were associated with reduced average hippocampal volume ($\beta = -.334$, $P < .01$, adjusted $R^2 = 0.410$, $F(4,53) = 9.225$, $P < .0001$). Similarly, higher CSF Ng/BACE1 was associated with reduced average amygdala volume ($\beta = -.234$, $P < .05$, adjusted $R^2 = 0.369$, $F(4,53) = 9.230$, $P < .0001$). When the amyloid-positive subjects (SCD and MCI, n = 31) were analyzed separately, higher CSF Ng/BACE1 was significantly associated with reductions in both hippocampal ($\beta = -.388$, $P < .05$, adjusted $R^2 = 0.350$, $F(4,27) = 5.175$, $P < .01$) and amygdala volumes ($\beta = -.420$, $P < .01$, adjusted $R^2 = 0.502$, $F(4,27) = 8.814$, $P < .0001$) (Effects are depicted in Fig. 2). No other associations between CSF biomarkers or *APOE*-ε4 carrier status and MTL volumetry were found. Significant regression coefficients are shown in Table 3. No overall significant differences in average hippocampal or amygdala volumes between groups were found. Please see Table 2 for details.

### 3.4. CSF biomarkers and APOE-ε4 in relation to baseline cognitive performance

We found a significant inverse relationship between higher CSF Ng/BACE1 and lower performance in CERAD learning T-score ($R^2 = 0.71$, $F(1,70) = 5.321$, $\beta = -.266$, $P < .05$); CERAD recall T-score ($R^2 = 0.97$, $F(1,70) = 7.535$, $\beta = -.312$, $P < .01$); and TMT-A T-score ($R^2 = .057$, $F(1,70) = 4.153$, $\beta = -.238$, $P < .05$) (effect shown in Fig. 3). Moreover, when controlling for age ($\beta = -.124$, $P = .31$), we found that higher Ng/BACE1 ($\beta = -.258$, $P < .05$) also was associated with lower scores on the MMSE (adjusted $R^2 = 0.78$, $F(2,70) = 4.044$, $P < .05$).

No relationships between baseline cognitive measures and *APOE*-ε4 carrier status or other CSF biomarkers were demonstrated. Statistically significant relationships were only found when analyzing the entire sample and are summarized in Table 3.

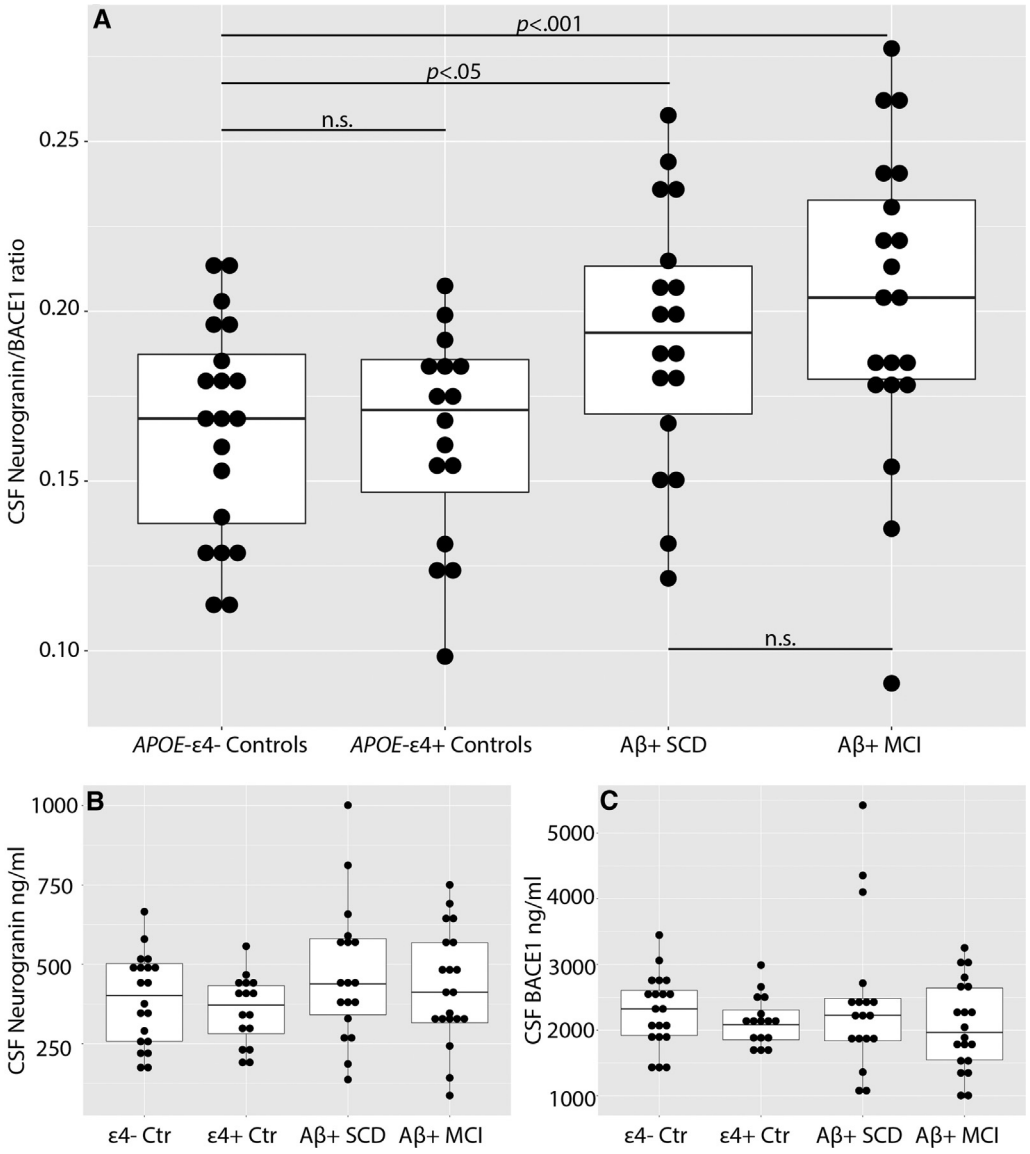


Fig. 1. Ng/BACE1 ratio (A), CSF Ng level (B), and CSF BACE1 level (C) between groups. Abbreviations: CSF, cerebrospinal fluid; Ng, neurogranin; BACE1, β-site amyloid precursor protein-cleaving enzyme 1; Ctr, controls; *APOE*-ε4+/−, apolipoprotein E4 allele positive or negative; Aβ+, CSF amyloid pathology; SCD, subjective cognitive decline; MCI, mild cognitive impairment. Horizontal brackets showing contrast comparisons for CSF Ng/BACE1 only (A). Significant results ($P < .05$) or nonsignificant results (n.s.) are shown.

### *3.5. Baseline CSF biomarkers and APOE-ε4 carrier status predicting change in cognitive performance at 2-year follow-up*

Lower baseline CSF Ng/BACE1 levels predicted practice effects (i.e., showing improved performance between baseline and follow-up), whereas increasing levels predicting less improvement and finally a decline between assessments in both CERAD learning T-score ($R^2 = 0.124$, $F(1,40) = 5.646$, $\beta = -.352$, $P < .05$) and MMSE ($R^2 = 0.97$, $F(1,42) = 4.426$, $\beta = -.312$, $P < .05$). A similar result was also obtained for Ng measured separately but only relating to the CERAD learning T-score ($R^2 = 0.104$, $F(1,40) = 4.622$, $\beta = -.322$, $P < .05$). Similarly, CSF t-tau significantly predicted cognitive decline in CERAD learning ($R^2 = 0.170$, $F(1,40) = 8.217$, $\beta = -.413$, $P < .01$) (effects are illustrated in Fig. 3). No relationships between 2-year cognitive change, *APOE*-ε4 carrier status, or other baseline CSF biomarkers were found. Significant relationships between baseline biomarkers and follow-up cognitive performance are summarized in Table 3.

## 4. Discussion

To our knowledge, this is the first study showing that Ng/BACE1 level is increased already at a preclinical stage of AD. Ng/BACE1 levels were equally increased in both

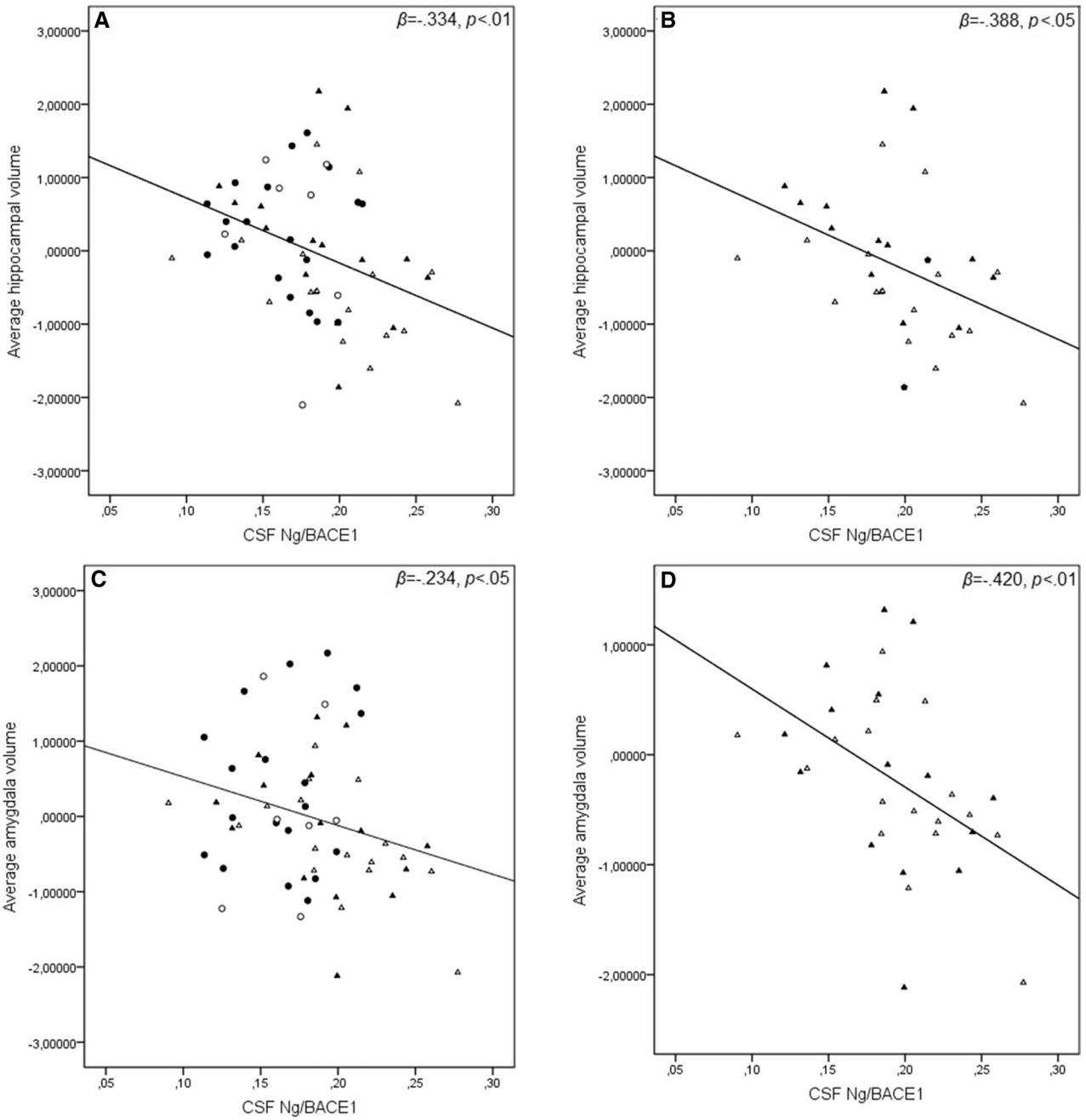


Fig. 2. CSF Ng/BACE1 in relation to medial temporal lobe volumetry. Average hippocampal (A & B) and amygdala volumes (C & D). Medial temporal lobe volumes are adjusted for age, gender, and MRI scanner variant. Open circles = *APOE*-ε4+ controls. Closed circles = *APOE*-ε4− controls. Open triangles = MCI with amyloid plaques. Closed triangles = SCD with amyloid plaques. Abbreviations: CSF, cerebrospinal fluid; Ng, neurogranin; BACE1, β-site amyloid precursor protein-cleaving enzyme 1; *APOE*-ε4+/−, apolipoprotein E4 allele positive or negative; SCD, subjective cognitive decline; MCI, mild cognitive impairment.

Aβ+ MCI and SCD groups compared with controls, and no difference in Ng/BACE1 levels between *APOE*-ε4 +/− controls were found. Increased Ng/BACE1 level was the only marker related to baseline hippocampal and amygdala volumes in our sample. Concordantly, the Ng/BACE1 level was the only biomarker associated with poorer baseline performance in both baseline CERAD learning and memory recall, as well as attention/psychomotor speed (TMT-A) and global cognitive function (MMSE).

Furthermore, when analyzing available 2-year follow-up cognitive scores, we found that lower baseline Ng/BACE1 levels predicted practice effects in the CERAD learning subtest at follow-up (i.e., showing improved performance) and increasing ratios predicted less

Table 3
Regression coefficients between biomarkers, MTL volumes, and cognitive tests at baseline and difference in T-score at 2-year follow-up

| Variable | CSF Ng | CSF BACE1 | CSF Ng/BACE1 | CSF t-tau | CSF $A\beta_{1-42}$ | *APOE*-ε4 allele positivity |
|---|---|---|---|---|---|---|
| | Biomarker and MTL measures entire sample (n = 57)/Aβ+ SCD and Aβ+ MCI (n = 30) | | | | | |
| Amygdala | */* | */* | $\beta = -.234$† / $\beta = -420$†; **$P < .05$ / $P < .01$** | */* | § | */* |
| Hippocampus | */* | */* | $\beta = -.334$† / $\beta = -.388$†; **$P < .01$ / $P < .05$** | */* | § | */* |
| | Biomarkers and Baseline cognitive tests (N = 74)/Biomarkers and cognitive change at 2-year follow-up (n = 42) | | | | | |
| MMSE | */* | */* | $\beta = -.258$‡ / $\beta = -.312$; **$P < .05$ / $P < .05$** | */* | § | */* |
| CERAD learning T-score | */$\beta = -.322$; */ **$P < .05$** | */* | $\beta = -.266$ / $\beta = -.352$; **$P < .05$ / $P < .05$** | */$\beta = -.413$; */ **$P < .01$** | § | */* |
| CERAD recall T-score | */* | */* | $\beta = -.312$/* **$P < .05$**/* | */* | § | */* |
| TMT-A T-score | */* | */* | $\beta = -.238$/* **$P < .05$**/* | */* | § | */* |
| TMT-B T-score | */* | */* | */* | */* | § | */* |

Abbreviations: CSF, cerebrospinal fluid; Ng, neurogranin; MTL, medial temporal lobe; BACE1, β-site amyloid precursor protein-cleaving enzyme 1; *APOE*-ε4+/−, apolipoprotein E 4 allele positive or negative; SCD, subjective cognitive decline; MCI, mild cognitive impairment; CERAD, the Consortium to Establish a Registry for Alzheimer's Disease word list test; MMSE, Mini Mental State Examination; TMT, trail-making test; MRI, magnetic resonance imaging.

*Nonsignificant result.

†Model includes age, gender, and MRI scanner variant as covariate.

‡Model includes age as covariate.

§Not performed at baseline due to study design selection bias.

improvement and finally a decline in CERAD word list–learning ability. This relationship was also shown for CSF Ng measured separately, supporting previous findings [2,4]. Although a similar result was obtained with CSF t-tau as the baseline predictor, an inspection of the scatter plot indicated that the regression model may have been biased by a few subjects with extreme baseline CSF total tau values. 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 [38]. In contrast, Ng/BACE1 levels 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 Ng/BACE1 level 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 [7]. Moreover, although a higher Ng/BACE1 level was related to lower MMSE at baseline and decline at follow-up both in our previous [21] and present studies, Ng/BACE1 level 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 level. 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 [39,40].

BACE1 and neurogranin have predominantly presynaptic [9,10] and postsynaptic roles, and neurogranin, in particular, is linked to the dendritic spine NMDA $Ca^{2+}$-Calmodulin second messenger complex [8]. Although synapse degeneration *per se* is not disease specific, the link between Aβ oligomerization, NMDA disruption, and spine $Ca^{2+}$-dysregulation [11,13] may confer an AD specificity to the Ng/BACE1 ratio marker and point to a postsynaptic Aβ-linked disease mechanism. This further strengthens the suggestion that NMDA antagonists may be protective in AD [41]. In this scenario, enhanced synaptotoxic polymerization of Aβ-peptides in *APOE*-ε4 SCD and MCI cases will have a more rapid synaptic loss due to increased levels of synaptotoxic Aβ fibrils [11,14,15]. Although *APOE*-ε4 carrier status did not significantly relate to medial temporal volumes or cognition in our sample, a large majority of the Aβ+ SCD and MCI cases (28 of 37) had at least one *APOE*-ε4 allele. Moreover, *APOE*-ε4 carriers with amyloid plaques had higher CSF Ng/BACE1 levels than noncarriers with plaques (data not shown). The Ng/BACE ratio was shown to increase with A/T/N-classified AD biomarker severity (i.e., moving from normal CSF

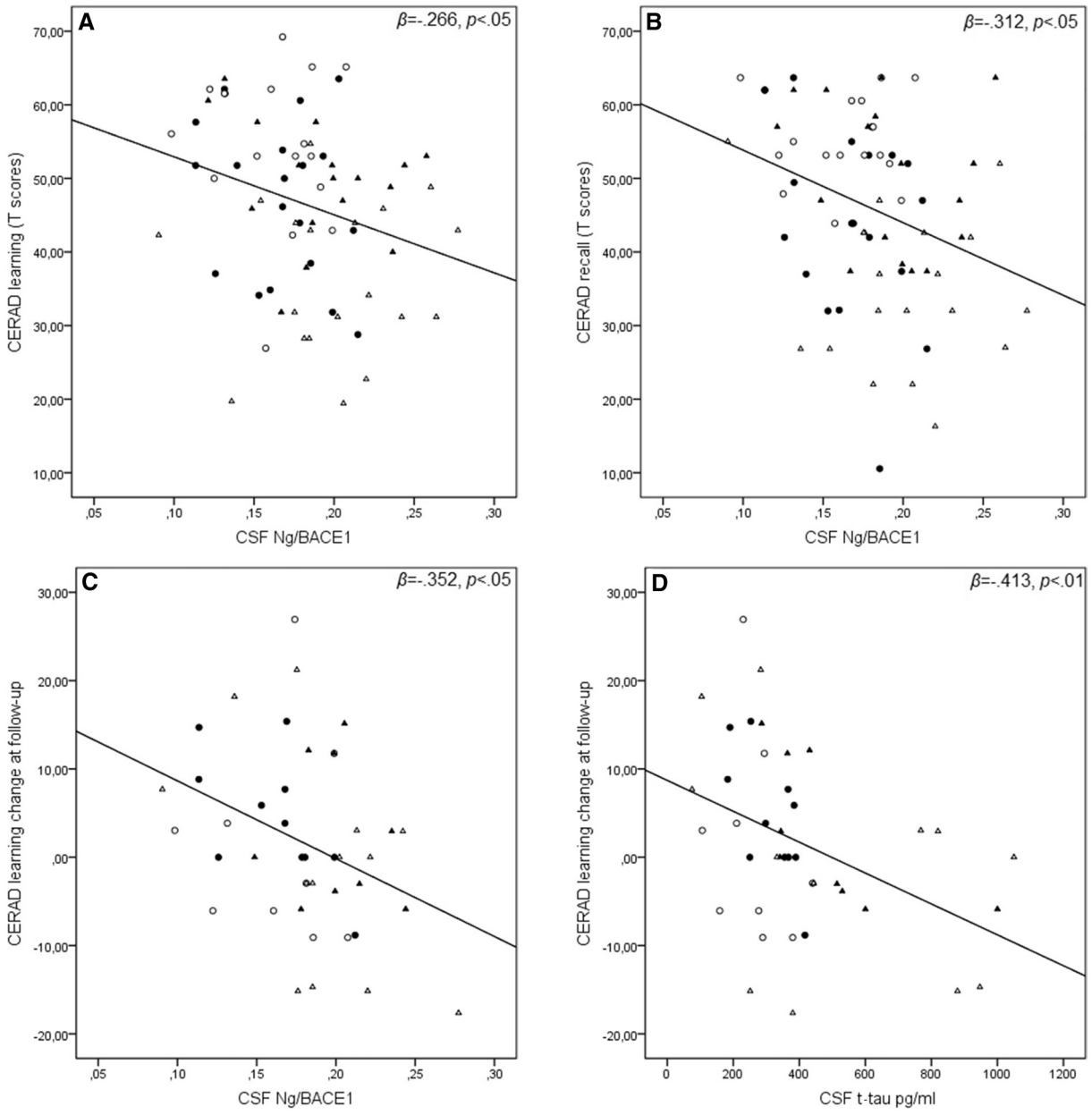


Fig. 3. CSF Ng/BACE1 and CSF t-tau in relation to baseline and 2-year follow-up CERAD learning and memory recall tests. CSF Ng/BACE1 and baseline CERAD subtest T-scores (A & B). CERAD Learning T-score change at follow-up CSF Ng/BACE1 (C) and CSF t-tau (D). Open circles = *APOE*-ε4+ controls. Closed circles = *APOE*-ε4− controls. Open triangles = MCI with amyloid plaques. Closed triangles = SCD with amyloid plaques. Abbreviations: CERAD, the Consortium to Establish a Registry for Alzheimer's Disease word list test; CSF, cerebrospinal fluid; Ng, neurogranin; BACE1, β-site amyloid precursor protein-cleaving enzyme 1; *APOE*-ε4+/−, apolipoprotein E4 allele positive or negative; SCD, subjective cognitive decline; MCI, mild cognitive impairment.

toward amyloid plaques combined with markers of neurodegeneration and neurofibrillary tangles) [19]. An increase was also observed for both CSF BACE1 [20] and Ng [21] separately, supporting previous findings indicating a link to neurodegeneration. Though *APOE*-ε4 could enhance Ng/BACE1-related pathology through its interaction with Aβ [11,14,15], a larger material with more *APOE*-ε4− and Aβ+ SCD and MCI cases will be needed to establish ε4-allelic effects.

Both the link to cognitive measures and strong associations to volume reductions in pertinent MTL structures lend further support to a putative role of Ng/BACE1 as a biomarker for Alzheimer-related synaptic loss. CSF Ng/BACE1 level was similarly increased in the Aβ+ MCI and SCD groups, thus the SCD cases may harbor an active disease state, including progressive synaptic loss, experienced as a SCD that has yet to reach the threshold for clinical impairment.

Some limitations of this study need to be addressed. First, care must be taken in interpreting these findings 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). This may explain why we did not show an expected association between CSF Ng and hippocampal volume in our sample [2,4] or expected between-group differences in MTL atrophy in amyloid-positive subjects [42,43]. Second, although the National Institute on Aging and Alzheimer's Association (NIA-AA) [28] recommends an MCI cutoff value of between −1 and −1.5 SD below the mean, we opted for a stringent cutoff at ≤−1.5 SD which can impact SCD/MCI group classification. However, cognitive performance in the SCD group was similar to that in the control group in our study, indicating that the SCD group's cognitive performance was within the normal range. Finally, we did not include Aβ-negative SCD or MCI cases or explore potential differences between homozygote and heterozygote *APOE*-ε4 carriers to other APOE genotypes; both of which we plan to explore in subsequent articles.

### 4.1. Conclusions

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 Ng/BACE1 is significantly increased relative to controls in amyloid-positive subjects with SCD. These results warrant further studies investigating the role of Ng/BACE1 in the AD pathogenesis, potentially reflecting synaptic pathology due to an Aβ-linked disease mechanism. Although NMDA antagonists have been suggested to be protective [36], the present findings suggest that such intervention guided by an early Ng/BACE1 increase might be useful.

## Acknowledgments

The project was funded by Norwegian Research Council, NASATS (Dementia Disease Initiation), and the JPND (APGeM) and funding from the regional health authorities (Helse Sør-Øst and Helse Nord). This article represents independent research (part) funded by the National Institute for Health Research (NIHR) Biomedical Research Center at South London and Maudsley NHS Foundation Trust and King's College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR, or the Department of Health. The authors thank Marianne Wettergren (Akershus University hospital, Norway) for essential help with CSF analysis and Erna Utnes (Akershus University hospital, Norway) for assistance with data collection and handling.

## RESEARCH IN CONTEXT

1. Systematic review: Synapse loss occurs early in Alzheimer's disease (AD). Increased CSF neurogranin (Ng) is related to synapse loss and β-site amyloid precursor protein-cleaving enzyme 1 (BACE1) is involved in presynaptic amyloid-β precursor protein metabolism. Previously, we found that an increased Ng/BACE1 ratio predicted cognitive decline in predementia AD. This ties in with the findings linking reduced beta-amyloid clearance to postsynaptic spine affection in early AD. Here, we investigate CSF Ng/BACE1 level as a preclinical marker of synapse loss in AD.
2. Interpretation: We found higher CSF Ng/BACE1 levels in preclinical and predementia AD related to reduced hippocampal volume and memory function at baseline and cognitive decline at follow-up. These results lend support to Ng/BACE1 as an early marker of synaptic loss in AD, which is sensitive also for preclinical changes.
3. Future directions: A high Ng/BACE1 ratio may point to the AD-related damage of postsynaptic spines. If confirmed, this could indicate specific early intervention measures and show target engagement in intervention studies.

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THE CLINICAL NEUROPSYCHOLOGIST
https://doi.org/10.1080/13854046.2019.1574902



# Demographically adjusted CERAD wordlist test norms in a Norwegian sample from 40 to 80 years

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## ABSTRACT

**Background/Objective:** In recent years, several slightly younger cohorts have been established in order to study the preclinical and prodromal phases of dementia. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) wordlist memory test (WLT) is widely used in dementia research. However, culturally adapted and demographically adjusted test norms for younger ages are lacking.

**Method:** This paper investigates effects of age, gender and years of education on test performance and offers demographically adjusted norms for the CERAD WLT using a regression-based norming procedure for the age span 40–80 years based on healthy controls ($n = 227$) from the Norwegian "Dementia Disease Initiation" (DDI) ($n = 168$) and "Trønderbrain" ($n = 59$) cohorts. In order to evaluate normative performance, we apply the norms to an independent sample of persons diagnosed with mild cognitive impairment (MCI = 168) and perform 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:** Demographically adjusted norms for the CERAD WLT for ages 40–80 years based on a Norwegian sample are proposed. To our knowledge, this is the first normative study of this test to offer demographically adjusted norms for this age span.

## ARTICLE HISTORY

Received 5 November 2018
Accepted 22 January 2019

## KEYWORDS

MCI; Alzheimer's disease; neuropsychological tests; Norms; CERAD

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Supplemental data for this article is available online at https://doi.org/10.1080/13854046.2019.1574902.

## Introduction

The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) was founded to standardize procedures for the evaluation and diagnosis of patients with Alzheimer's Disease (AD) (Morris et al., 1989). The instruments developed by CERAD have been widely used and have been translated into several languages and validated within different cultural contexts (Fillenbaum et al., 2008). Its clinical utility has mainly focused on detecting mild cognitive impairment (MCI) and AD with dementia. Normative data are primarily developed for elderly cohorts (Beeri et al., 2006; Fillenbaum et al., 2005; Schmidtke & Hermeneit, 2008; Sotaniemi et al., 2012; Welsh et al., 1994). However, it has been shown that AD develops over 10–15 years before clinical cognitive impairment is evident (Bateman et al., 2012; Jack et al., 2018). Thus, a major focus in dementia research has shifted to the asymptomatic or preclinical stages (Sperling et al., 2011). In order to capture disease events in these stages, several slightly younger cohorts have been established (Soldan et al., 2013; Weiner et al., 2015). To capture individual cognitive decline and significant treatment effects at these stages, more narrow and culturally adapted norms for cognitive tests including CERAD subtests may need to be established. Recently, Hankee et al. (2016) proposed norms for the CERAD Word list test (WLT) for younger and middle-aged adults based on an American sample. These norms are primarily provided for younger persons (<55 years) and are adjusted for either age or education. However, as learning and memory are influenced by age, education and gender (Beeri et al., 2006; Liu et al., 2011) correction for additional demographic factors may be necessary in order 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.

The use of discrete norms (e.g. capturing the normative performance of a certain demographic) requires an adequate sample size in order to ensure that the reference group is a representative sample of the population distribution. When adjusting for several demographic characteristics such as gender, age and education, the sample size requirement increases dramatically (Oosterhuis, van der Ark, & Sijtsma, 2016). Moreover, norm-based performance may increase substantially by moving from one age category to the next, due to distinct differences between normative reference groups (e.g. moving from a 54–59 year group to 60–65 year group) (Zachary & Gorsuch, 1985). Continuous norms employing regression-based norming procedures offer a possible solution to these issues by requiring 2.5–5.5 times smaller sample size (Oosterhuis et al., 2016) while offering the possibility for continuous adjustment of multiple demographic variables such as age, gender and years of education.

We propose norms adjusted for age, gender and years of education based on a regression-based norming procedure using the normative performance of healthy controls ($n = 227$) aged 40–80 years from two established prospective Norwegian cohorts, investigating preclinical and prodromal dementia. 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 calculate *T* scores in a group of Norwegian speaking patients ($n = 168$) aged 40–80 years previously diagnosed with MCI from the Dementia Disease

Initiation (DDI) cohort and fit regression models to confirm that the norms reliably adjust for demographic variables when applied to an independent sample.

## Methods and materials

The DDI cohort employs a standardized protocol for participant selection and assessment and includes healthy controls, as well as participants with subjective cognitive decline (SCD) and MCI. Healthy control subjects were recruited primarily from spouses of symptomatic participants (SCD or MCI), and secondarily from volunteers responding to advertisements in media, newspapers, or news bulletins. The cohort was recruited between 2013 and 2018. Criteria for inclusion were age between 40 and 80 years and a native language of Norwegian, Swedish, or Danish. Exclusion criteria were brain trauma or disorder, including clinical stroke, dementia, severe psychiatric disorder, severe somatic disease that might influence cognitive functions, or intellectual disability or other developmental disorders. At the time of analysis, 168 fluent Norwegian speakers, 166 (98.8%) Norwegian native and 2 (1.2%) Swedish native healthy controls were included ($n = 168$). A subset of these controls ($n = 23$) were missing total sum scores on the CERAD WLT 20-item recognition subtest (total true positives and true negatives) due to the scoring of only true positives (10-item score). These subjects were removed from further analysis, leaving a total of $n = 145$ with total sum scores on this test. The healthy controls were all able to complete the CERAD WLT in accordance with test instructions (detailed below). In order to evaluate the regression-based norms in an independent sample, we included 168 fluent Norwegian speakers, 167 (99.4%) native Norwegian and 1 (0.6%) native Danish participants from the DDI cohort previously diagnosed with MCI. MCI cases from the Trønderbrain cohort was not included in this analysis since the sample was smaller compared to the MCI sample from the DDI cohort, and slightly different cognitive tests and diagnostic algorithms for classification of MCI diagnosis were used (Berge et al., 2016; Fladby et al., 2017). In the DDI cohort, MCI was determined according to published criteria (Albert et al., 2011; Petersen, 2004), and cases were classified as cognitively impaired when obtaining a score $\leq$1.5 standard deviation below the normative mean on CERAD word list delayed recall (using norms from Sotaniemi et al. (2012)), Visual Object and Space Perception Battery (VOSP) silhouettes (Warrington & James, 1991), Trail Making Test B (TMT-B) or Controlled Oral Word Association test (COWAT) (Heaton, Miller, Taylor, & Grant, 2004). 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 was $>$0.5 (Petersen, 2004). For further description of the DDI cohort and methods, see Fladby et al. (2017).

The Trønderbrain 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. At the time of analysis, 59 healthy controls with Norwegian native language aged 57–80 years were included. They were all able to complete the CERAD WLT in accordance with test instructions (detailed below). The CERAD recognition subtest was not administered and normative data for this subtest is therefore

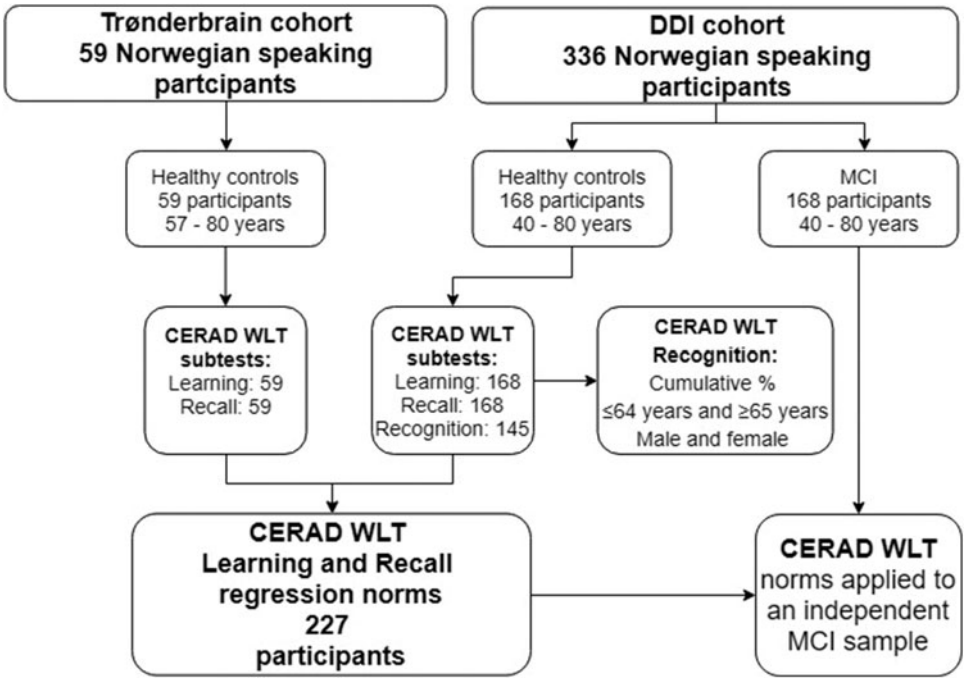


Figure 1. Flowchart depicting an outline of the participant inclusion process from the Trønderbrain and DDI cohorts and workflow of the paper.

only available from the DDI cohort. Exclusion criteria were a present psychiatric or malignant disease (i.e. currently undergoing treatment for cancer), use of anticoagulant medication, or high alcohol consumption. For further description of the Trønderbrain cohort and methods, see Berge et al. (2016). An outline of the participant inclusion process is depicted in Figure 1.

Participants were recruited and assessed from several university hospitals across Norway. This covered a representative sample of residents in cities and nearby rural areas from northern Norway (Troms and Finnmark, $n = 10$), mid-Norway ($n = 66$, Trondheim), south-east Norway ($n = 58$, Akershus/Oslo), south-west Norway ($n = 43$, Bergen and $n = 50$, Stavanger/Haugesund).

### *CERAD word list test version and administration*

The CERAD WLT was translated to Norwegian by Liv Barnett in 2004 using the 10-item word list from the original CERAD test description. The Norwegian version was sourced from CERAD (Gerda Fillenbaum, PhD) at the Center for the Study of Aging and Human Development, USA (Fillenbaum et al., 2008). The following words (and Norwegian translation) were used: Queen (Dronning), Grass (Gress), Arm (Arm), Cabin (Hytte), Pole (Stokk), Shore (kyst), Butter (Smør), Engine (Motor), Ticket (Billett), Letter (Brev). The test was administered by healthcare professionals (medical doctors, nurses and psychologists) at the different sites. Beforehand, all had received training in the administration of the CERAD WLT by the DDI projects senior/chief neuropsychologist, prof. Erik Hessen.

The CERAD WLT learning score was obtained from the sum of three 10-word learning trials yielding a maximum score of 30. After 10 min, participants were asked to recall the words from the 10-word learning trials, yielding a maximum score of 10. We did not record intrusions or perseverations. Finally, a recognition trial comprising 20 words was administered where 10 of the words were distractors, and 10 words were target items from the 10-word learning list. This yielded a maximum score of 20 (10 true positive and 10 true negative responses). However, total sum of false positives was not recorded for the present project.

The following procedure was used when administering the CERAD WLT (here translated from Norwegian to English):

### *CERAD WLT trial 1*

Verbal instruction to participant: "I will now show you 10 words consecutively. Read every word out loud. Afterward I will ask you to recall the presented words"

Instruction to the test administrator: Show every word for 2 s, if the participant is not able to read the word, please read the word out loud for the participant.

### *CERAD WLT trial 2–3*

Verbal instruction to participant: "I will now show you 10 words consecutively once more. Read every word out loud. Afterward I will ask you to recall the presented words"

Instruction to the test administrator: Same as for trial 1 instruction detailed above.

### *CERAD WLT Recall trial (presented 10 min after trial 3 administration)*

Verbal instruction to participant: "A little while ago, I asked you to learn and remember a list of words, which you read out loud for me one at a time. Now, I want you to recall as many of those 10 words as you can remember".

### *CERAD WLT Recognition trial*

Verbal instruction to participant: "Now I will read out loud all 10 words from the list in addition to some other words that were not on the list. I want you to reply "Yes" if you recognize a word from the list you read out loud for me, and "No", if it is a word that did not belong to the list you read out loud".

## *Statistical analysis*

Multiple linear regression analyses with age, gender and years of education as predictors were fitted to model CERAD WLT performance in healthy controls ($n = 227$). Models were also fitted with interaction terms (age squared) to test for non-linear relationships between age and test performance (i.e. improving with younger age, and declining with older age). However, the inclusion of this interaction term did not add to the overall explained variance (adjusted $R^2$) of the regression models. Thus, only linear terms were included in our models. Overall estimates of the models (adjusted $R^2$, $F$ value, $p$ value), and relative contributions for individual predictors ($\beta$, partial $R^2$, $p$ value) are reported. Since the DDI and Trønderbrain cohorts employ slightly different

Table 1. Cumulative percentiles for the CERAD WLT recognition subtest.

| | Non-geriatric (≤64 years) | | | Geriatric (≥65 years) | | |
|---|---|---|---|---|---|---|
| | Male $n = 33$ | Female $n = 52$ | Total $n = 85$ | Male $n = 26$ | Female $n = 34$ | Total $n = 60$ |
| Age range (mean) | 45–64 (57.8) | 40–64 (54.8) | 40–64 (55.9) | 65–80 (70.4) | 65–78 (69.6) | 65–80 (69.9) |
| Mean (SD) | 19.2 (1.1) | 19.8 (0.6) | 19.6 (0.9) | 19.0 (1.7) | 19.2 (1.2) | 19.1 (1.4) |
| Range | 16.0–20.0 | 17.0–20.0 | 16.0–20.0 | 13.0–20.0 | 15.0–20.0 | 13.0–20.0 |
| 2% | 16 | 17 | 17 | 13 | 15 | 13 |
| 5% | 17 | 19 | 17 | 14 | 17 | 15 |
| 10% | 17 | 19 | 19 | 16 | 18 | 17 |
| 25% | 19 | 20 | 19 | 19 | 19 | 19 |
| 50% | 20 | 20 | 20 | 20 | 20 | 20 |
| 75% | 20 | 20 | 20 | 20 | 20 | 20 |
| 90% | 20 | 20 | 20 | 20 | 20 | 20 |

*Notes. n:* Number of participants; SD: standard deviation.

criteria for inclusion and exclusion, this variable was assessed in separate regression models to assess a potential between-cohort bias. However, no significant bias of cohort was found.

## Norming procedure

Due to a marked ceiling effect, the CERAD recognition subtest failed to produce a normal distribution of test scores, which is required for the regression-based norming procedure. Our data suggest that age and gender are the strongest demographic contributors to test performance. Thus, percentiles split by gender are provided for non-geriatric (e.g. 40–64 years, $n = 85$) and geriatric (65–80 years, $n = 60$) groups (Table 1).

As shown in Figure 1, the CERAD learning and recall raw test scores from the healthy control group were used to develop demographically adjusted regression-based norms. Methods and rationale used for regression-based norming in this paper are similar to procedures employed by Heaton et al. (2004), Testa, Winicki, Pearlson, Gordon, and Schretlen (2009) and Parmenter, Testa, Schretlen, Weinstock-Guttman, and Benedict (2010). We first normalized the control groups raw test scores 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 (Table 2). We regressed the resulting scaled scores on age, gender and education. 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. To derive normative information and calculate demographically adjusted *T* scores for each participant in the MCI group, we used the multiple regression equations derived from this analysis (Table 3) to compute the participants predicted scaled scores. The participants scaled score, derived from the healthy control group's normal distribution (Table 2) was subtracted from the regression equation predicted scaled score for each participant. The resulting discrepancy score was divided by the standard deviation of healthy control group's residuals (Table 3) to yield a standardized z score, which was then converted to a *T* score.

Lastly, multiple linear regression analyses with age, gender and years of education as predictors were fitted to the DDI MCI group's CERAD WLT learning and recall *T*

Table 2. Raw score to scaled score conversions.

| Scaled score | CERAD learning | CERAD recall |
|---|---|---|
| 3 | | ≤1 |
| 4 | ≤13 | 2 |
| 5 | 14–15 | 3–4 |
| 6 | 16–17 | |
| 7 | 18 | 5 |
| 8 | 19 | 6 |
| 9 | 20 | |
| 10 | 21–22 | 7 |
| 11 | 23 | 8 |
| 12 | 24 | |
| 13 | 25 | 9 |
| 14 | 26 | 10 |
| 15 | 27 | |
| 16 | 28 | |
| 17 | ≥29 | |

Table 3. Normative regression models for CERAD WLT Learning and Recall subtests.

| Variable | Predictor | *B* | Standard error *B* | *T* | SD residual |
|---|---|---|---|---|---|
| CERAD Word list Learning | | | | | |
| | Constant | 14.195 | 1.600 | 8.871 | 2.54678 |
| | Age | −0.100 | 0.020 | −5.024 | |
| | Gender | 1.060 | 0.354 | 2.992 | |
| | Education | 0.113 | 0.053 | 2.125 | |
| CERAD Word list Recall | | | | | |
| | Constant | 13.756 | 1.571 | 8.759 | 2.51947 |
| | Age | −0.093 | 0.020 | −4.752 | |
| | Gender | 1.176 | 0.350 | 3.359 | |
| | Education | 0.107 | 0.052 | 2.045 | |

*Notes. B*: unstandardized regression coefficient; *T*: the t test statistic; SD: standard deviation.

score distributions to confirm adequate adjustment of demographic variables in an independent sample. All analyses were performed in the Statistical Package for Social Sciences (SPSS) version 25.

## Norm calculator implementation

To facilitate the adoption and usage of the proposed norms in the clinic, we have developed a norm calculating tool that computes the regression equations. The functionality is simple and straightforward. To obtain both learning and recall *T* Scores, the user needs to enter valid demographic (age, gender and years of education) values and respective raw scores obtained from the tests. The tool is implemented as a self-contained HTML/Javascript webpage, available at (https://uit.no/ressurs/uit/cerad/cerad-calc.html), and released as open source at https://bitbucket.org/apgem/cerad-calc under Apache License, version 2.0.

## Ethics

Both DDI and the Trønderbrain projects had been approved by the regional medical research ethics committees. Before taking part in the study, participants gave their written informed consent. 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.

## Results

### Demographic characteristics in healthy controls compared to the MCI group

The demographic characteristics of the healthy control group ($n = 227$) are summarized in Table 4 and compared to pertinent demographics of the independent MCI sample ($n = 168$) using summary independent $t$ tests. While the MCI group obtained significantly lower raw scores in both CERAD WLT learning ($p < .0001$) and recall subtests ($p < .0001$), the groups were similar with regards to mean age and years of education.

### Impact of demographics on the CERAD WLT performance within the healthy control group

Multiple regression analysis showed advancing age to predict decline in performance ($\beta = -.310$, partial $R^2 = .101$, $p < .0001$), whereas years of education ($\beta = .126$, partial $R^2 = .018$, $p < .05$) and female gender ($\beta = .201$, partial $R^2 = .046$, $p < .001$) was associated with increased performance on the CERAD word list learning subtest (adjusted $R^2 = .164$, $F$ (3,223 = 15.751, $p < .0001$). Similarly, younger age ($\beta = -.279$, partial $R^2 = .083$, $p < .0001$), higher education ($\beta = .139$, partial $R^2 = .022$, $p < .05$) and female gender ($\beta = .208$, partial $R^2 = .048$, $p < .01$) predicted better performance on the CERAD word list recall subtest (adjusted $R^2 = .151$, $F$ (3,224) = 14.418, $p < .0001$). No collinearity was observed between the predictor variables in any of the models (Variance Inflation Factor <1.1).

### Regression-based norms and scoring instruction

Table 3 shows the regression models based on the healthy controls to derive norms for the CERAD learning and recall subtests. All models include coefficients to adjust for age, gender (male = 0, female = 1) and years of education.

Table 4. Demographics, raw scores and $T$ scores of the healthy controls and MCI group.

| Variables | Healthy controls $n = 227$ Test scores/demographics | MCI group $n = 168$ Test scores/demographics | Sig. |
|---|---|---|---|
| Age | 63.1 (8.6) [40–80] | 64.4 (9.4) [40–80] | n.s* |
| Gender (% females) | 62 | 54 | |
| Years of education | 14.2 (3.2) [7–23] | 13.6 (3.4) [7–22] | n.s* |
| CERAD WORD LIST (30) LEARNING | | | |
| Raw score | 21.5 (3.3) | 16.9 (4.3) | **$p < .0001$*** |
| $T$ score | 50.0 (10.0) | 38.7 (10.1) | **$p < .0001$*** |
| CERAD WORD LIST (10) RECALL | | | |
| Raw score | 7.2 (2.0) | 4.3 (2.7) | **$p < .0001$*** |
| $T$ score | 50.0 (10.0) | 37.4 (11.4) | **$p < .0001$*** |

*Notes.* $n$: Number of participants; MCI: mild cognitive impairment; Sig.: significance tests; $p$: $p$ value; $T$: $T$ score; n.s.: non-significant result. Results are presented as mean (standard deviation) [range] except for gender which is characterized by female percentage. *Summary independent $T$ tests. Significant $p$ values are shown in bold.

The raw test scores of the MCI group ($n = 168$) were converted to $T$ scores using the following stepwise procedure: (1) Look up the scaled score for a given subtest in Table 2. (2) Use the regression coefficients found in Table 3 to obtain a predicted scaled score [constant + individual age(coefficient for age)+individual gender(coefficient for gender)+individual years of education(coefficient for education)]. (3) Then, subtract the actual scaled score from the predicted scaled score and divide it by the standard deviation of the residual (Table 3) to obtain a standardized $z$ score which may be converted to a $T$ score [$T = z(10)+50$]. For example, the $T$ score calculation for a 50-year-old male with 8 years of education with a scaled score of 10 (Table 2) on CERAD learning: $14.195 + 50(-0.100)+8(0.113)+0(1.060)=10.099$. The difference between actual (10) and the predicted scaled score (10.099) is $-0.099$. Divided by the standard deviation of the healthy control groups residuals (2.54678) gives a $z$ score of $-0.039$ which equates to a $T$ score of 49.61.

### *Evaluation of demographic adjustment in the MCI group*

Multiple regression models with age, gender and years of education as predictors were non-significant in the MCI group for both regression derived normative CERAD learning $T$ scores (adjusted $R^2$=.009, $F$ (3,165 = 1.531, $p$=.208) and CERAD recall $T$ scores (adjusted $R^2$=.005, $F$ (3,165 = 1.293, $p$=.279), indicating adequate adjustment of pertinent demographics when norms are applied to an independent sample.

## Discussion

In this study, we have developed demographically adjusted norms for the CERAD WLT aimed at ages 40–80 years in a Norwegian sample. To our knowledge, this is the first normative study offering CERAD WLT norms aimed at this age interval, adjusted for the effects of both age, gender and education. As expected, increasing age had the largest impact on CERAD word list performance, followed by smaller effects of education and gender. These findings are in line with previous studies showing declining performance with increasing age (Sotaniemi et al., 2012; Welsh et al., 1994), a positive effect of educational attainment on performance (Beeri et al., 2006) and a female advantage on tests of verbal list learning tests or vocabulary (Beeri et al., 2006; Heaton et al., 2004; Liu et al., 2011). Thus, the regression-based norms were developed adjusted for these demographics. Healthy controls were recruited from two different prospective Norwegian cohorts. No between-cohort bias on test performance was found.

Regression-based norming procedures require stringent methodological criteria to be met (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. Furthermore, age is non-linearly related to many cognitive functions, including memory performance, with increasing capacity in early life superseded by a slow decline in later life (Hartshorne & Germine, 2015). We accounted for non-linearity by introducing an age squared term in our regression models. 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 the normative data provided by Hankee et al. (2016) provide age adjusted normative data for younger ages (primarily for ages 35–55 years), comparisons with the proposed regression-based norms would not be appropriate due to the insufficient coverage of older ages (40–80 years). Similarly, the norms by Sotaniemi et al. (2012) originally employed in the DDI study are based on an older cohort with lower educational level compared to the participants enrolled in the DDI study (Kirsebom et al., 2017). In summary, this prompted the need to provide adequate norms covering ages for both earlier, and later stages of disease development and progression. We therefore opted to assess normative performance in an independent sample of MCI cases drawn from the DDI study covering both younger and older patients. We found that the regression-based norms successfully adjusted for age, gender and years of education in an independent sample of MCI cases. Furthermore, 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 therefore be due to factors largely independent of normal aging, gender differences and educational level.

Interestingly, while years of education did predict higher performance on both CERAD WLT learning and recall subtests, 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 has obtained a university degree (bachelor's degree or higher) with more than 15 years of education in total. As such, the relatively high educational level observed in our study could be a cultural bias, which could influence estimated normative performance on neuropsychological tests (Hayden et al., 2014; Heaton et al., 2004). These norms were developed in a Norwegian sample. However, they should be adequate for other Scandinavian countries which share similarities in culture, education and language. While all of our healthy controls were fluent in Norwegian, two participants (0.9%) were Swedish natives, who had lived in Norway most of their adult lives. Similarly, one Norwegian speaking Danish native (0.4%) was included in the MCI sample. The English CERAD WLT items were translated to Norwegian using back translation procedure to ensure accuracy.

A noteworthy finding using the predictions offered from the regression norms is 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. This indicates that the CERAD WLT may be too easy for these individuals. Thus, in order to detect longitudinal change in cognitive proficiency due to degenerative brain disease, we recommend the addition of a more challenging wordlist test such as the Rey Auditory Verbal Learning Test (RAVLT) (Schmidt, 1996) for younger individuals.

A limitation of this study is the missing scores on the CERAD WLT recognition memory test. In addition, this subtest shows a marked ceiling effect, and does not produce a normal distribution of test scores required for regression-based norming. Our data indicate that age and gender had the highest influence on normative performance. Thus, normative performance on this test is shown by providing cumulative percentile ranks for geriatric ($\geq$65) and non-geriatric ($\leq$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. Finally, regression-based norms may not be as easy and familiar to use for clinicians compared to conventional discrete norms. In order to overcome this, we offer a free web-based intuitive normative calculator (supplementary file 1/https://uit.no/ressurs/uit/cerad/cerad-calc.html).

## Conclusion

We propose demographically adjusted regression-based norms for the CERAD WLT, based on healthy controls from the Norwegian DDI and Trønderbrain cohorts. The norms are linearly adjusted for the effects of gender, age and education between the ages of 40 and 80 years, with an educational attainment between 7 and 23 years.

## Acknowledgments

We thank Svein Ivar Bekkelund, Kjell-Arne Arntzen, Kai Müller, Torgil Riise Vangberg, Claus Albretsen, Mari Thoresen Løkholm, Ida Harviken, Line Sæther, Erna Utnes, Marianne Wettergreen, Berglind Gisladottir, Marit Knapstad, Reidun Meling, Mats Berg Aslaksen, Synnøve Bremer Skarpenes and Elin Margrethe Solli for clinical examinations and essential help with the project.

## Funding

This work was supported by the Norwegian Research Council (Dementia Disease Initiation) under Grant No. 217780; Helse Sør-øst, NASATS (Dementia Disease Initiation) under Grant No. 2013131; Helse Midt under Grant No. 46028700 and Helse Nord under Grant No. HNF1401-18.

## Data availability statement

The data that support the findings of this study are available on request from the corresponding author, B-E.K. The data are not publicly available due to restrictions e.g. their containing information that could compromise the privacy of research participants.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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