Risk factors and predictors of dementia and cognitive impairment

Neergaard, Jesper

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Risk factors and predictors of dementia and cognitive dysfunction
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Supervisors

Associate Professor Susanne Brix Pedersen, PhD

DTU Bioengineering

Technical University of Denmark

Associate Professor Christopher Workman, PhD

DTU Bioengineering

Technical University of Denmark

Head of Neurodegenerative Diseases Kim Henriksen, PhD

Biomarkers & Research

Nordic Bioscience A/S

Chief Executive Officer Morten Asser Karsdal, PhD

Biomarkers & Research

Nordic Bioscience A/S

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Jesper Skov Neergaard, ID: 136536

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“Prediction is very difficult,
especially when it is about the future”

Niels Bohr, Danish physicist

To Louise, Erika and Kamille

Preface

This thesis is based on work carried out at Nordic Bioscience A/S and the Department of Biotechnology and Biomedicine at the Technical University of Denmark from November 2013 to February 2017 (excluding August 2015 to November 2015). All research has been anchored in the Prospective Epidemiological Risk Factor Study (PERF), a Danish population-based cohort of postmenopausal women. The project was divided in two parts. First, recruitment, data collection and data management for the follow-up study (PERFII). This part required 14 months of full-time involvement and ended up with enrolling a total of 2,103 elderly Danish women, of whom I personally met 800 – an exciting, challenging and truly educational experience. Alongside the data collection the application for register-linkage was submitted and approved. The second part included the data analysis and manuscript preparation.

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Jesper Skov Neergaard
Risk factors and predictors of dementia and cognitive dysfunction

Papers included in the thesis

Paper I

JS Neergaard*, K Dragsbæk*, SN Kehlet, HB Hansen, G Hansen, I Byrjalsen, P Alexandersen, LM Lindgren, A Bihlet, BJ Riis, JR Andersen, P Qvist, MA Karsdal, and C Christiansen. **Cohort Profile: The Prospective Epidemiological Risk Factor (PERF) Study**. International Journal of Epidemiology. 2016. doi: 10.1093/ije/dyw251.

Paper II

JS Neergaard, K Dragsbæk, HB Hansen, K Henriksen, C Christiansen, and MA Karsdal. **Late-Life Risk Factors for All-Cause Dementia and Differential Dementia Diagnoses in Women: A Prospective Cohort Study**. Medicine (Baltimore). 2016;95(11):e3112.

Paper III

JS Neergaard, K Dragsbæk, C Christiansen, HB Nielsen, S Brix, MA Karsdal and K Henriksen. **Metabolic Syndrome, Insulin Resistance and Cognitive Dysfunction: Does your metabolic profile affect your brain?** In revision Diabetes, submitted November 2016.

Paper IV

JS Neergaard, K Dragsbæk, C Christiansen, MA Karsdal, S Brix and K Henriksen. **Objective Cognitive Impairment and Progression to Dementia in Women: The Prospective Epidemiological Risk Factor Study**. The Journal of Prevention of Alzheimer's Disease. 2017:In press.

Paper V

JS Neergaard, K Dragsbæk, C Christiansen, MA Karsdal, S Brix, and K Henriksen. **Two novel serum biomarkers measuring degradation of tau are associated with dementia: a prospective study**. Under review in Alzheimer's Research & Therapy, submitted January 2017.

*JS Neergaard and K Dragsbæk contributed equally to the work

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Finally, **Louise**, for all your support, for sharing life with me and last but not least for sharing the greatest gift of all with me, **Erika and Kamille**. This thesis is dedicated to all my three girls.

Abbreviations

A4	The Anti-Amyloid Treatment in Asymptomatic Alzheimer’s Study
AD	Alzheimer's Disease
APOE	Apolipoprotein E gene
APP	Amyloid Precursor Protein
AUC	Area under the Receiver-Operating Characteristics curve
Aβ	Amyloid beta
BBB	Blood-Brain-Barrier
CAIDE	Cardiovascular Risk Factors, Aging, and Dementia study
CFT	Category Fluency Test
CI	Confidence Interval
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CT	Computed Tomography
DLB	Dementia with Lewy bodies
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
EOAD	Early Onset Alzheimer's Disease
FDG	Fluorodeoxyglucose
FTD	Frontotemporal dementia
ICD-10	International Classification of Diseases and Related Health Problems 10th Revision
LOAD	Late Onset Alzheimer's Disease
MCI	Mild Cognitive Impairment
MRI	Magnetic Resonance Imaging
NFT	Neurofibrillary tangles
NIA-AA	National Institute on Aging and the Alzheimer's Association
NPR	Danish National Patient Register
OD	Other/Unspecified Dementia
PERF	Prospective Epidemiological Risk Factor Study
PET	Positron Emission Tomography
PTMs	Post-Translational Modifications
RCD	Danish Register of Causes of Death
RCTs	Randomized Controlled Trials
SBT	Short Blessed Test
SNAP	Suspected non-Alzheimer’s Disease pathophysiology
SPECT	Single Photon Emission Computed Tomography
VaD	Vascular Dementia

Content

Summary

The greying of the world population has led to what was previously referred to as the “silent” epidemic of our century, namely dementia. The epidemic is primarily driven by an epidemiological transition, where prolonged longevity and declining fertility rates have led to increasing proportions of older people in the total population. Dementia and cognitive impairment are by far the leading causes of disability and in particularly the need for care among older people. Surprisingly there has been much less investment in dementia research, given its burden. Consequently, Alzheimer’s disease, being the most prevalent dementia type, is the only cause of death among the top 10 killers in the United States that cannot be prevented, cured, or even delayed. The knowledge of risk and protective factors is therefore especially important for the development of prevention strategies, as prevention by risk factor intervention, is considered the key to a better control of the epidemic. Women outlive men on average, however they have poorer health status. Moreover, women have an elevated risk of dementia. This clearly justifies an increased focus on dementia specifically for women. In the development of new disease modifying interventions there has been a devastating low rate of success in the area of dementia. Resources have therefore been directed at identifying preclinical stages of dementia-related diseases as this is considered the optimal “window” for intervention. Identification of subjects with preclinical disease and subsequent high likelihood of progression are therefore an indisputable prerequisite for the success of future drugs. Here, biomarkers play a crucial role, as the pre-symptomatic diagnosis will rely on these. Hence, advances in biomarkers, especially non-invasive blood-based biomarkers, are required to ensure that the new drugs are tested on the right patients at the right time.

The aims of this thesis were: *i)* to identify risk factors for all cause and differential dementia diagnoses, *ii)* to identify risk factors associated with progression from normal cognition to dementia within the follow-up period and *iii)* to evaluate the possible utility of two novel serological biomarkers of truncated tau as predictors of incident dementia. This was investigated using data from the Prospective Epidemiological Risk Factor (PERF) study, a population-based prospective cohort study on 5,855 elderly Danish women initially enrolled between year 1999 and 2001 with a follow-up examination of 2,103 of the women in year 2013-2014.

We aimed at identifying risk factors for incident dementia and its subtypes in chapter 4. With special focus on a range of metabolic risk factors we investigated how these factors were related to cognitive dysfunction at the follow-up visit (chapter 5). These studies found that Body Mass Index (BMI) in the overweight range and physical activity were associated with lower risk of dementia (Chapter 4), while increasing age, history of depression, insulin resistance (using the

homeostasis model assessment index) and elevated fasting plasma glucose increased the risk of dementia or cognitive dysfunction (chapter 4 or Chapter 5, respectively).

In chapter 6 we specifically aimed at assessing the risk of progression to dementia in subpopulation(s) of women with signs of mild cognitive deficits and further to investigate the cognitive courses from baseline to follow-up (reverse trajectory, stable, and progressive) including a risk-profile specifically associated with progression. We found that the degree of cognitive impairment at baseline (single versus multiple domains) was an important predictor of dementia and in subjects with subtle objective cognitive impairment physical inactivity, elevated total cholesterol and a history of depression were associated with progression to dementia or severe cognitive impairment.

In chapter 7, we evaluated the possible utility of two novel serological biomarkers of truncated tau as predictors of incident dementia in women. We found that high levels of Tau-A and Tau-C were associated with lower risk of dementia and Alzheimer's disease. Tau-C gave a very modest increase in the area under the curve (AUC) in a 5-year prediction horizon as compared to a reference model with age and education.

Finally, we summarised our results in a nomogram, a simple tool for prediction of dementia tailored for individual risk prediction. This illustrates the applicability of such findings for dementia risk screening (chapter 8). Overall, many of the identified risk factors are considered modifiable and therefore provide further evidence that prevention strategies could be a way to counteract the otherwise poor future prospects for dementias in the ageing population. Also, we show that the risk factors and blood-based tau biomarkers may be useful in screening and thereby early identification of individuals at-risk for dementia, one of the most persisting needs in dementia drug development.

Resume

Verdens befolkning ældes, hvilket har medført dét, der tidligere blev omtalt som vores århundredes "tavse" epidemi; nemlig demens. Epidemien er primært drevet af en demografisk forskydning, hvor en højere middellevealder og faldende fødselsrater har medført en stadigt stigende andel af ældre i den samlede befolkning. Demens og kognitiv svækkelse er de primære årsager til invaliditet og især behov for pleje blandt ældre mennesker, men overraskende nok har der, på trods af dets byrde, været signifikant færre investeringer i demens forskning. Alzheimers sygdom som er den hyppigste form for demens er som en konsekvens heraf, den eneste dødsårsag blandt de 10 hyppigste dødsårsager i USA, som ikke kan forebygges, helbredes, eller blot forsinkes. Viden om risikofaktorer er derfor specielt vigtigt for udviklingen af forebyggelsesstrategier, da disse anses for at være nøglen til en bedre kontrol af epidemien. Kvinder lever i gennemsnit længere end mænd, de har dog en dårligere helbredstilstand. Desuden har kvinder en forøget risiko for demens. Dette berettiger et øget fokus på demens specielt hos kvinder. Der har været en meget lav succesrate i udviklingen af nye sygdomsmodificerende behandlinger på demens området. Ressourcerne er derfor nu blevet rettet mod identifikation af præklinisk demens. Dette sygdomsstadie betragtes af mange, som det optimale tidspunkt for opstart af sygdomsmodificerende behandlinger. Identifikation af personer med præklinisk sygdom og en efterfølgende stor sandsynlighed for progression er derfor en forudsætning for fremtidige lægemidlers succes. Biomarkører har her en afgørende rolle, da den præ-symptomatiske diagnose vil afhænge af disse. Udvikling af især non-invasive blod-baserede biomarkører skal derfor sikre, at de nye lægemidler testes i de rette patienter på det rette tidspunkt.

Formålene med dette projekt var: i) at identificere risikofaktorer for demens og undertyper heraf, ii) at identificere risikofaktorer forbundet med progression i opfølgningsperioden og iii) at vurdere potentialet af to nye serologiske biomarkører som prædiktorer for udvikling af demens. Dette blev undersøgt ved hjælp af data fra det Prospektive Epidemiologiske Risikofaktor (PERF) studie, en prospektiv kohorteundersøgelse af 5855 ældre danske kvinder oprindeligt inkluderet mellem årene 1999 og 2001, med en opfølgende undersøgelse på 2103 af kvinderne i årene 2013-2014.

Vi undersøgte hvilke risikofaktorer, der var associeret med generel demens og undertyper af demens in kapitel 4. Med særligt fokus på en række af metaboliske risikofaktorer, undersøgte vi hvordan disse faktorer var relateret til kognitiv dysfunktion ved den opfølgende undersøgelse (kapitel 5). Disse studier viste, at Body Mass Index (BMI) i det overvægtige interval samt fysisk aktivitet var associeret med en lavere risiko for demens (kapitel 4), samtidig var stigende alder, en nuværende eller tidligere depression, insulinresistens (målt ved hjælp af HOMA-IR indekset)

og et forhøjet faste glukose i blodet associeret med en øget risiko for udvikling af demens eller kognitiv dysfunktion (kapitel 4 eller kapitel 5).

Vi havde i kapitel 6 til formål at undersøge risikoen for progression til demens hos en subpopulation af kvinder, der ved inklusionen viste tegn på mild kognitiv svækkelse samt yderligere at undersøge hvorledes deres kognitive funktion havde udviklet sig fra baseline til follow-up. Herunder identificerede vi en risikoprofil specifikt forbundet med progression. Vi fandt, at graden af kognitiv svækkelse ved inklusionen (et enkelt versus flere kognitive domæner) var en vigtig prædikator for fremtidig demens og hos personer med mild kognitiv svækkelse var fysisk aktivitet, forhøjet total kolesterol og en tidligere eller nuværende depression forbundet med progression til demens eller svær kognitiv svækkelse.

I kapitel 7 vurderede vi to nye serologiske biomarkørers potentiale til identifikation af fremtidig demens hos kvinder. Vi fandt, at høje niveauer af Tau-A og Tau-C var forbundet med lavere risiko for demens og Alzheimers sygdom. Sammenlignet med en referencemodel indeholdende alder og uddannelsesniveau gav Tau-C en beskeden stigning i arealet under kurven (AUC) i en 5-års forudsigelseshorisont.

Til sidst opsummerede vi vores resultater i et nomogram, som er et simpelt værktøj, som baseret på den enkelte patients risikoprofil, anvendes til at forudsige demens. Nomogrammet illustrerer anvendeligheden af vores resultater i relation til demens screening (kapitel 8). Samlet set konkluderer vi, at mange af de identificerede risikofaktorer kan betragtes som modificer bare, hvilket giver yderligere bevis for, at forebyggelsesstrategier kan være en måde at påvirke de ellers dystre fremtidsudsigter for demenssygdomme i den aldrende befolkning. Desuden viser vi, at risikofaktorer og blod-baserede biomarkører kan være nyttige i screening og dermed tidlig identifikation af demens, hvilket er et af de mest presserende behov i udviklingen af ny demens medicin.

1

Introduction

1. Introduction

Dementia is a syndrome that describes a wide range of symptoms that occur when the brain is affected by certain conditions. Dementia can be grouped in reversible and irreversible dementia disorders. The reversible dementia disorders are most often drug induced, caused by hormonal imbalance or vitamin deficiencies and are out of scope in this thesis. The irreversible dementia disorders are progressive, degenerative disorders that are affecting memory and other cognitive functions to the extent that they interfere with a person's daily life and activities. The most common types of irreversible dementia include Alzheimer's disease (AD), vascular dementia (VaD) and mixed dementia, particularly the combination of AD and VaD.

Cognitive impairment is used as a broad term describing impairment in any one (or all) of the cognitive domains assessed by objective cognitive performance irrespective of the underlying cause. This thesis deals with cognitive impairment and all-cause dementia in general and the major types of dementia including AD, VaD and other/unspecified dementias (OD). The following sections will describe the causes, symptoms and underlying mechanisms of the dementia disorders, with emphasis on AD.

1.1 Dementia, a threat to Global Health and Aging

Advances in medicine and socioeconomic development have made one of humanity's greatest achievements, namely: prolonged longevity [1]. The rise in life expectancy accompanied by declining fertility rates is now driving an epidemiological transition increasing the proportion of older people in the total population. In Europe alone, the elderly population (>65 years) is estimated to double from 88 to 153 million by 2060 and the fastest growing segment of the population will be those aged 80 and older tripling in number from 24 to 60 million [2]. This demographic shift is associated with increased prevalence of chronic diseases and as it is also accompanied by prolonged survival, it will put a large pressure on healthcare systems [3]. Maintaining a healthy life is therefore of outmost importance. While women outlive men on average, they have poorer health status [4,5] and this clearly justifies an increased focus on ageing, specifically of women.

One of the most daunting and costly consequences of ever-longer life expectancies is dementia. Dementia and cognitive impairment are by far the leading causes of disability and in particularly need for care among older people worldwide, thus it has been estimated that the health and social care costs for dementia exceed costs of other chronic diseases like cancer, cardiovascular disease and stroke [6]. Unfortunately, there has been much less investment in dementia research, given its burden, compared with research in cancer and cardiovascular disease. In Denmark,

women account for more than 2/3 of the total number of people living with dementia [7], and dementia is the second leading cause of death in women [8].

In 2015, *Alzheimer's Disease International* estimated that 46.8 million people were living with dementia worldwide. They projected the number to nearly triple by 2050 reaching 131.5 million people worldwide [6]. In Denmark, approximately 84,000 people were living with dementia in 2015. The number was estimated to increase with 80% reaching more than 150,000 by 2040 [7]. The 2015 World Alzheimer's Report states that the incidence of dementia doubles with every 6.3-year increase in age, from 3.9 per 1000 person-years at age 60-64 to 104.8 per 1000 person-years at age 90+ [6].

1.2 Nosology of Dementia Disorders

1.2.1 Diagnosis and Classification

The concept of dementia and its classification has developed on the basis of accumulating evidence of clinicopathological entities and presumed etiological factors. Two major diagnostic classification systems exist and are used for diagnosis of dementia. The WHO's International Classification of Diseases and Related Health Problems 10th Revision (ICD-10) and the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM 5). Further, the National Institute on Aging and the Alzheimer's Association (NIA-AA) proposed new diagnostic criteria for dementia, AD and mild cognitive impairment (MCI) in 2011 [9,10], and a working group also proposed diagnostic criteria for preclinical AD. Preclinical AD refers to an early disease stage where pathological changes in the brain can be detected using biomarkers [11]. Alongside the NIA-AA also an International Working Group proposed similar research diagnostic criteria for AD. Like the NIA-AA criteria it defines three stages of AD: preclinical AD, prodromal AD (MCI due to AD in the NIA-AA criteria) and AD dementia [12]. There are differences on how the stages are conceptualized however this will not be elaborated any further in this thesis. The diagnostic guidelines outline several cognitive stages ranging from normal cognition to dementia, as illustrated in figure 1.

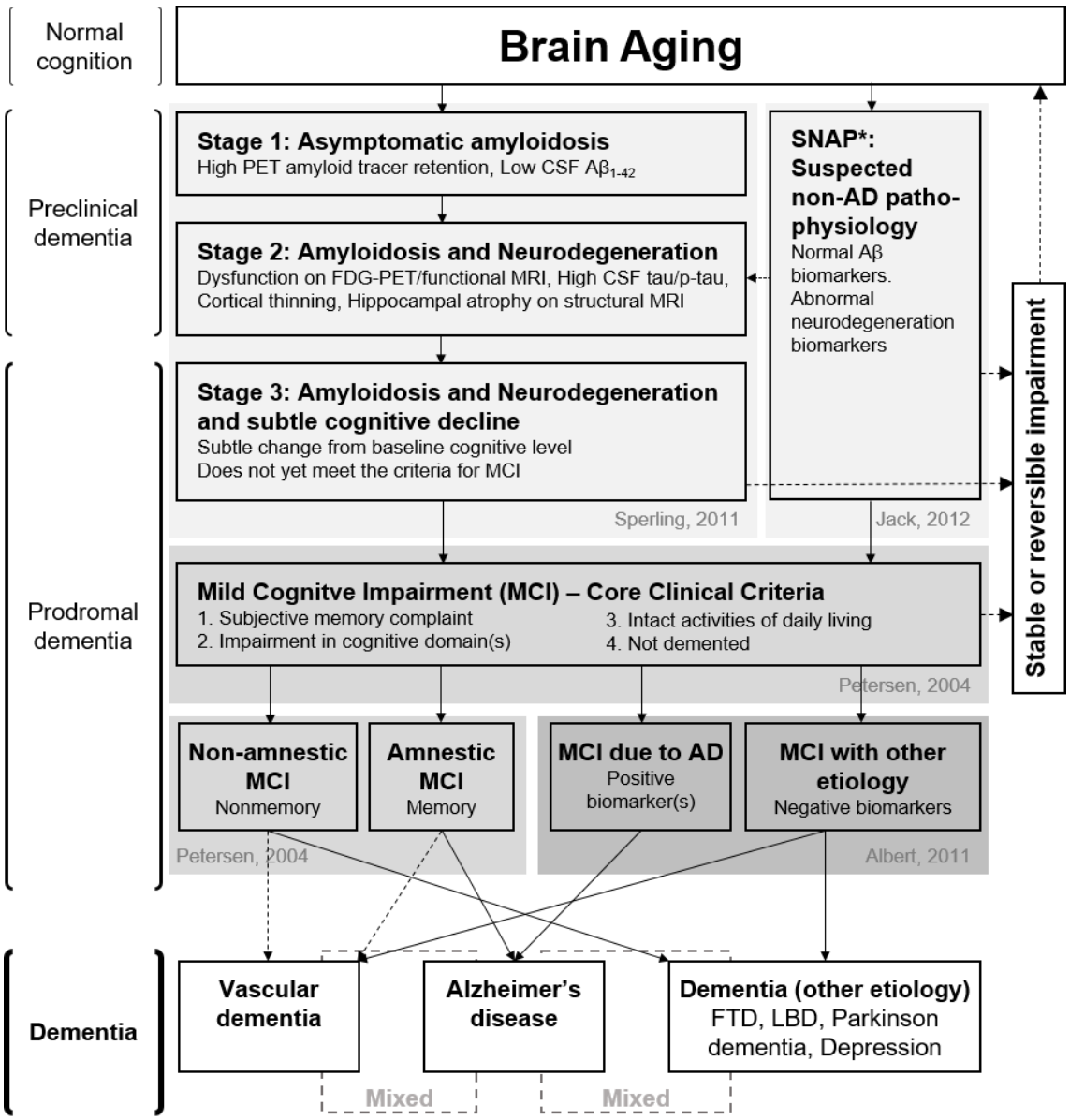

Figure 1: Overview of the cognitive stages from normal cognition to dementia. The overview is based on the diagnostic criteria for preclinical dementia suggested by Sperling et al. [11] and the core clinical criteria for mild cognitive impairment suggested by Petersen et al.[13] together with the revised MCI classification by Albert et al.[10]. The figure was made with modification from [14]. The concept of SNAP was introduced by Jack et al.[15]. *In this figure, SNAP includes common amyloid-negative neurodegenerative conditions like cerebrovascular disease, hippocampal sclerosis and the preclinical brain lesions of FTD and LBD. MCI: Mild Cognitive Impairment, CSF: Cerebrospinal fluid, PET: positron emission tomography, MRI: magnetic resonance imaging, FDG: Fluorodeoxyglucose.

The clinical diagnosis of dementia is based on the medical history, a neuropsychological test battery and a thorough clinical examination of symptoms. In addition, there are certain imaging biomarkers such as computed tomography (CT), magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT), positron emission tomography (PET) and

cerebral spinal fluid (CSF) biomarkers that may be used to support the clinical diagnosis [9]. These techniques are however mainly used in specialized clinics and for research purposes rather than in general practice. Detection of neuropathological lesions in the brain by autopsy is the gold standard for the diagnosis of dementia-related diseases [16].

1.2.2 Subtypes of Dementia

Traditionally there is a distinction between early and late onset dementia. AD is the most prevalent cause of dementia irrespective of the time of onset. Among younger people (<65 years of age) approximately one third of all dementia cases is caused by AD. Frontotemporal dementia (FTD), Dementia with Lewy bodies (DLB) and other types of dementia are relatively more prevalent among the younger as compared with the elder population [17]. It is estimated that AD accounts for up to 67% of all late onset dementia cases in women. The second most prevalent dementia type in women is VaD accounting for 15% followed by mixed dementia with 10% of the total number of dementia cases. The remaining 8% can be attributed to other types of dementia including: DLB (3%), FTD (1%) and Parkinson disease dementia (1%) [18]. The clinical and pathological features of the main subtypes of dementia are outlined in table 1.

Table 1: Overview of main dementia subtypes. The table was made with inspiration from [19]

	Subtypes of dementia			
	Alzheimer's Disease	**Vascular Dementia**	**Lewy Body Dementia**	**Frontotemporal Dementia**
Onset	Gradual	Acute or gradual	Insidious	Early Insidious
Progression	Gradual	Stepwise or gradual	Fluctuating	Rapid
Signs and Symptoms	Memory loss, language deficits, mood and personality changes	Memory loss, language deficits, dysarthria, emotional lability, decreased concentration	Depression, hallucinations, variability in terms of day to day symptoms	Poor judgement, social withdrawal, inappropriate behaviour
Regions of atrophy	General atrophy noted in the medial temporal lobe	Strokes, lacunar infarcts, white matter lesions	Generalized atrophy throughout	Frontal and temporal lobes
Pathologic features	Amyloid plaques Neurofibrillary tangles	Cerebrovascular disease	Lewy bodies	Pick bodies

Mixed dementia is used when more than one type of dementia occurs simultaneously in the brain. The most common mixed pathology is AD with VaD followed by AD with DLB [20]. The distinction between the subtypes of dementia may seem straightforward, however evidence from autopsy studies shows that differential diagnosis is very challenging. As outlined in figure 2, a previous study found that 77% of subjects with VaD and 66% of subjects with DLB also had AD pathology, while up to 50% of subjects with AD also had another pathology e.g. VaD or OD [21]. These

findings prove that different pathologies are often coexisting highlighting that mixed dementia might be more common than previously anticipated. This has been confirmed in another study where about half of the people with dementia had evidence of more than one co-existing pathology [20].

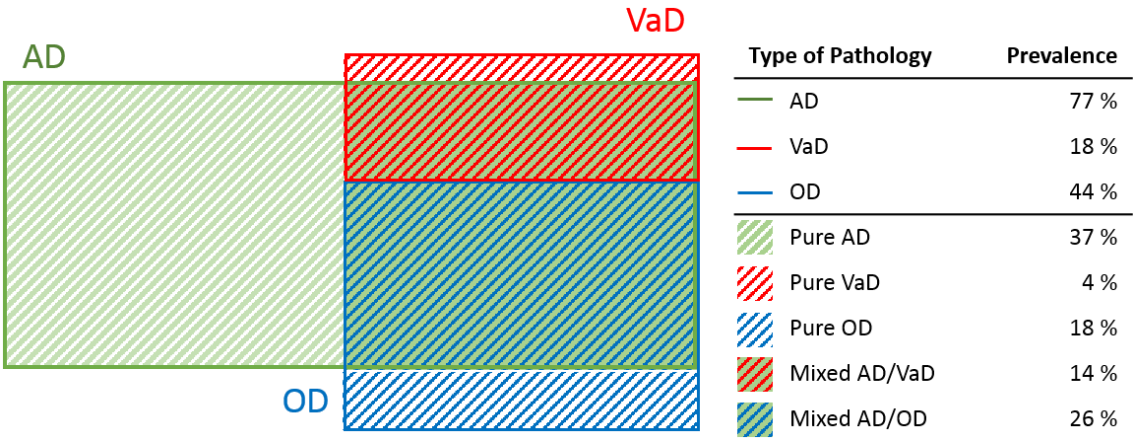

Figure 2: Venn diagram showing the diagnostic overlap of differential dementia diagnoses confirmed by autopsy. Diagram created based on findings by Barker et al.[21]. AD: Alzheimer's disease, VaD: Vascular dementia, OD: Other types of dementia (Frontotemporal dementia, Dementia with Lewy bodies, hippocampal sclerosis)

1.2.3 The disease continuum

The continuum of dementia-related diseases can cover a wide spectrum ranging from apparently normal cognition to advanced dementia. It progresses through several preclinical and clinical stages as illustrated in figure 3.

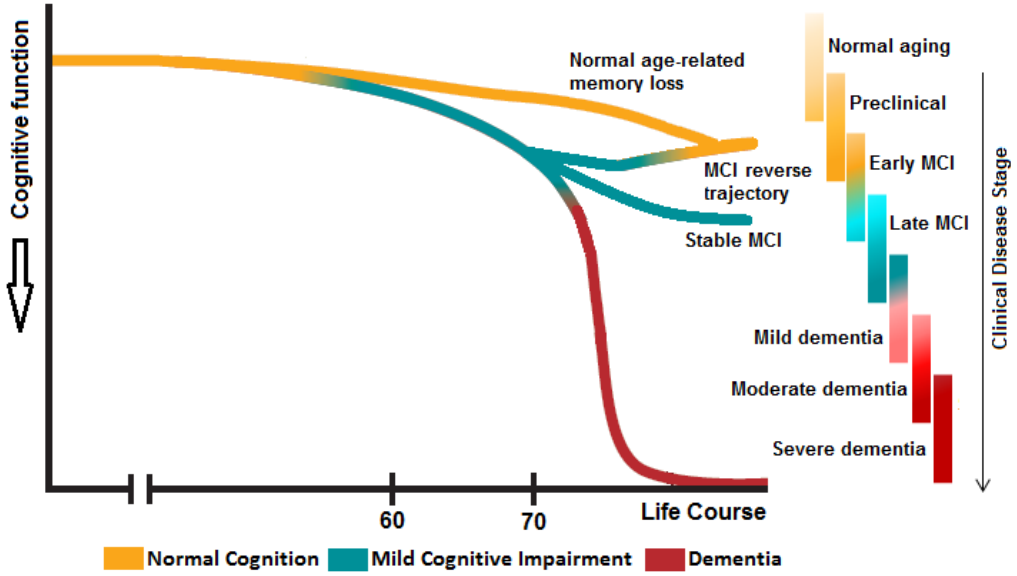

Figure 3: The disease continuum from normal cognition to severe dementia. Figure modified from [22].

1.2.3.1 Normal and Pathological Brain Aging

It is well-known that the brain shrinks in volume as we age [23]. The shrinkage is especially seen in areas related to learning, memory, planning, and other complex mental activities. Brain regions most commonly affected include the prefrontal cortex and the hippocampus. Brain regions that are also affected when a person develops a dementia disorder [24,25]. Alterations in neurotransmission by reduction of neurotransmitter levels and reduced blood flow are also found in the brain as a result of normal aging, but to a much lesser degree as compared to pathological brain aging [26]. Accumulation of pathological hallmarks of AD has been observed in about a third of very old people without dementia or cognitive impairment [27]. This evidence indicates that there is a certain degree of overlap between normal and pathological changes in the aging brain, making it difficult to determine when normal aging stops and pathological neurodegeneration begins.

1.2.3.2 The Preclinical stage

Dementia-related diseases, and AD in particular, are characterized by a long preclinical phase, where the pathological alterations in the brain are believed to begin decades before the clinical onset [28]. The general consensus is, that the preclinical stage provides the best opportunity for potential disease modifying interventions [11]. The recent research recommendations from the NIA-AA [11] approach a diagnostic guideline for preclinical AD, however, it is still lengths from clinical implementation. The recommendations contain a temporal framework with three stages defined from; biomarker evidence of amyloid-beta (Aβ) accumulation (stage 1), presence of one or more markers of neuronal injury (stage 2) and finally positivity on amyloid and neuronal injury markers combined with a subtle cognitive decline (stage 3) (figure 1). A concept named Suspected non-AD pathophysiology (SNAP) was later introduced by Jack et al. [15]. SNAP was defined by biomarker evidence of non-AD neurodegenerative processes (normal Aβ biomarkers, abnormal markers of neuronal injury.

1.2.3.3 Mild Cognitive Impairment

MCI has become the most widely used concept in research on early cognitive deficits. In MCI, the cognitive deficits are worse than would normally be expected for a healthy person of equivalent age, however not severe enough to interfere with activities in daily life [13]. It is well-known that MCI increases the risk of later developing dementia, and the condition is therefore often considered an intermediate stage between normal aging and the earliest features of dementia. Importantly, not all people with MCI progress; some people remain stable over time while others even return to normal levels [29] (figure 3). Due to this heterogeneity researchers are focusing on identifying people with MCI who are most likely to progress to dementia.

To qualify for an MCI diagnosis subjects should present with: *i)* a subjective cognitive complaint *ii)* objective cognitive impairment, *iii)* preserved activities of daily living, and *iv)* not demented [13]. According to the Petersen criteria [13], subjects that fulfil this core clinical criteria are then subdivided in four subgroups of MCI. If memory is impaired, subjects are characterised with amnestic MCI. Alternatively, if memory is not impaired, subjects are designated as having non-amnestic MCI. The presence or absence of impairment in multiple other cognitive domains further divide these subjects into amnestic or non-amnestic MCI with either single or multiple domain involvements [13]. The amnestic subtypes of MCI are believed to progress to AD if there is an underlying degenerative etiology. In contrast, non-amnestic MCI may progress to other types of dementia such as FTD if a single domain is affected or DLB if multiple domains are affected [29]. Both amnestic and non-amnestic MCI may precede VaD as illustrated in figure 1.

The revised MCI classification by Albert et al. in 2011 was outlined with the purpose of developing diagnostic criteria for the symptomatic predementia phase of AD. Subjects fulfilling the core clinical criteria for MCI are divided into two subgroups: MCI due to AD and MCI with other etiology. Additional information from imaging and CSF biomarkers determines whether a person with MCI has underlying AD pathology and thus is characterized with MCI due to AD [10].

1.2.3.4 The clinical stages of dementia

The initial clinical stage of dementia designated mild dementia is characterised by memory lapses that will affect daily life, such as forgetting words, misplacing things and problem-solving difficulties. As mild dementia progresses subjects will need more assistance in their daily life. The moderate stage of dementia implies personality and behavioural changes. There is increasing confusion and memory loss. The end stage of severe dementia is often manifested by loss of the ability to communicate, loss of physical capabilities and eventually death [30].

1.3 Risk Factors for Dementia

Dementia disorders are multifactorial disorders and the development is regulated by several environmental and genetic risk factors. The degree of inheritance and inheritance patterns varies considerably between different dementia disorders. It is well-known that genes play a role in the development of AD and FTD, while the impact of genes seems to be much smaller in the development of VaD and DLB. AD can be divided into a dominantly inherited familial form also referred to as early onset AD (EOAD) and a non-familial or “sporadic” form known as late onset AD (LOAD). The genetic predispositions are considered non-modifiable risk factors while the modifiable risk factors can be of demographic, behavioural, biomedical, environmental or social origin. Non-modifiable and modifiable risk factors can act independently but most often in

combination [31]. A huge body of research has been conducted in relation to risk factors for dementia and AD in particular.

1.3.1 Risk Factors across the lifespan - The importance of time

An important aspect in the study of risk factors is that risk factors may change over time [32], wherefore it is important to consider when the risk factor is assessed relative to the outcome. The relation between time and some of the most well studied risk factors is illustrated in figure 4.

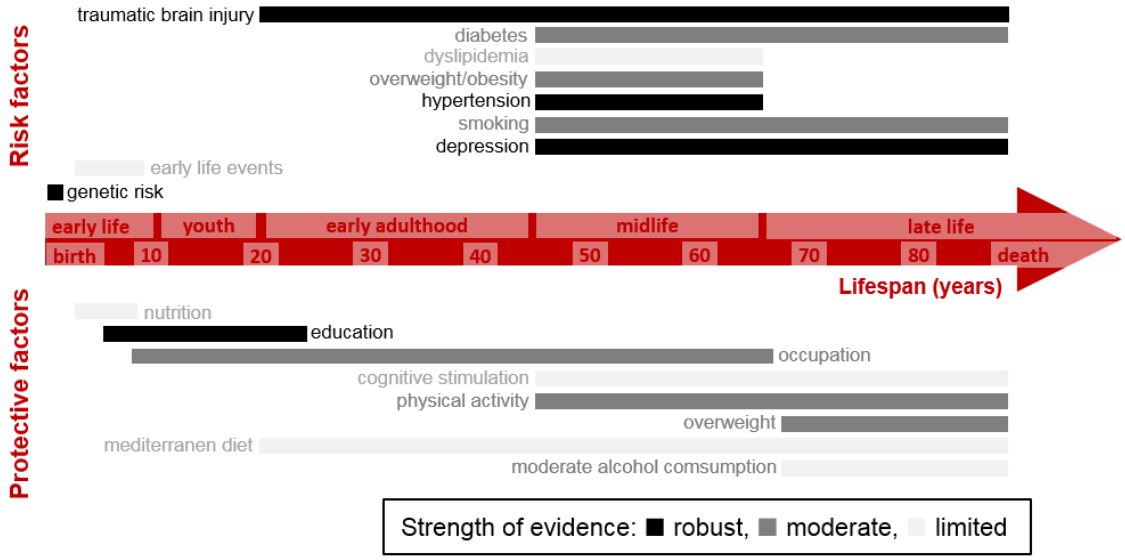

Figure 4: Risk factors for dementia across the lifespan. Strength of evidence is indicated on grey-scale with strongest evidence in black, moderate evidence in grey and limited evidence in light grey. Timeline was created based on information from [33,34]

It is obvious that genetic risks develop prenatally, while factors in early life such as certain life events and length of education have been linked to later risk of dementia [33]. Lifestyle and vascular risk factors have been found to impact the risk of dementia in mid- to late-life. Risk factors like hypertension and dyslipidaemia are most important in midlife while diabetes, depression and physical and mental activity affect the risk of dementia in both mid- and late-life [33].

1.3.2 Non-Modifiable Risk Factors

1.3.2.1 Age, gender and family history

The primary risk factor for dementia is advancing **age**. After the age of 65 the risk of dementia increases exponentially as it approximately doubles with every five years of ageing. Disproportionality has been observed in relation to **gender** and risk of dementia and most evidence suggests that women have an increased risk of AD, while men may be slightly more prone to develop VaD [34]. Subjects with a **family history** of dementia are also suspected to have a higher risk of dementia and AD although the evidence is limited [36,37].

1.3.2.2 Genetic factors

The dominantly inherited EOAD, accounting for up to five percent of all AD cases, is caused by mutations in one of **three deterministic genes** encoding for the amyloid precursor protein (APP), presenilin-1 and presenilin-2 on chromosome 21, chromosome 14 and chromosome 1, respectively [38–40]. Evidence also indicates the presence of other unidentified causative genes that remain to be identified, since EOAD cases without these known mutations have been observed [41]. FTD is also inherited in an autosomal dominant manner and one of the most common mutations in FTD is found in the *MAPT* gene on chromosome 17, encoding for tau.

Beside the deterministic genes, a range of **risk genes** have been linked to risk of AD. The apolipoprotein E gene (*APOE*) is the risk gene with the highest impact, and has been associated with both EOAD and LOAD. Specific variants of the *APOE*, namely the epsilon 4 alleles increase the risk of AD. Subjects that are heterozygous for epsilon 4 have a 3 times higher risk of AD while homozygosity increases the risk up to 15 times as compared to subjects with two copies of the epsilon 3 allele [42]. The epsilon 4 allele also affects the time of disease onset. The age of onset is lowered by approximately a decade from when a similar person, without this genotype, would have otherwise developed AD [43].

Several other genes have been linked to a greater risk of AD. The *CLU, CR1 and TREM2* genes involved in the clearance of Aβ and inflammation appear to be associated with AD however the impact and exact role have not been fully elucidated [44,45]. It has also recently been suggested that the *MAPT* gene may also play a role in relation to AD [46].

1.3.3 Modifiable Risk Factors

1.3.3.1 Vascular factors

Many cases of dementia and AD can be attributed to vascular risk factors such as **hypertension and diabetes**. It appears that a dose-response like relationship exists between the number of risk factors and subsequent risk of dementia and AD, where subjects with several concurrently occurring risk factors are at a particular high risk [47,48]. Diabetes and its associated conditions**; insulin resistance and metabolic syndrome** are associated with an increased risk of dementia [49,50]. Diabetes has been intensively studied and consistent evidence shows that the risk of dementia is increased on average between 50–100% for subjects with diabetes as compared to subjects without diabetes. The association is strongest for VaD compared to LOAD [51].

Hypertension in midlife, not late-life, increases the risk of dementia, and is generally stronger for VaD than with AD [52,53]. **Overweight and obesity** have previously been linked to dementia and AD in both midlife and late-life. A BMI in midlife indicating overweight or obesity has often been proposed to increase risk of developing dementia in later life [54,55]. In late-life several large

prospective cohort studies have shown a negative relationship between higher BMI and risk of dementia [56,57]. The most recent evidence strengthened this inverse association in late-life and suggests that a negative association does apply also in midlife [58]. Most studies on midlife **total cholesterol** show a positive association with risk of dementia, while the evidence on late-life total cholesterol most often reports negative or no association however there is a high degree of inconsistency in general [59].

1.3.3.2 Lifestyle factors

Smoking is associated with an elevated risk of dementia and cessation decreases the risk to that of never smokers [60]. There is a degree of dose-response relationship, suggesting that the higher amount of smoking, the greater the risk of developing dementia [61]. The evidence between **alcohol consumption** and risk of dementia is sparser. A J-shape relationship has been suggested, with moderate drinkers having a lower risk than abstainers and heavy drinkers, which was confirmed by a meta-analysis in the 2014 World Alzheimer's Report [33].

Leisure activities comprising **physical activity, mental activities and social engagement** have all been found to have protective effects in relation to development of dementia [62]. Physical activity may be associated with up to 40% lower risk of dementia, which is believed to be modulated partly through improved cardiovascular health [33]. Cognitive leisure activities during mid- or late-life have also been associated with lower risk of dementia in late-life [63]. Adherence to a **Mediterranean style diet** is associated with lower risk of developing dementia, AD in particular [64].

1.3.3.3 Other factors

A high level of **education** has consistently been associated with reduced risk of dementia. Recently, a critical threshold of completing more than 10 years of education was identified as an important mediator of the educational effect [65]. High level of education is known to be associated with a healthier lifestyle and this is assumed to explain part of the inverse relation between educational level and dementia. Another accepted explanation is the idea of the 'cognitive reserve', i.e. the ability to maintain a good cognitive performance despite brain pathology [66].

Late-life **depression** increases the risk of dementia and has been associated with both VaD and AD, with the strongest association with the former condition [67]. However, the causality has been questioned [68].

1.3.4 The need for further studies on risk factors for dementia

In dementia research, there are certain issues that may challenge the ability to establish true cause-effect relationships, where the non-arguable criterion in epidemiology is that the exposure has to precede the outcome in time. First, due to the insidious onset of dementia-related disorders it is challenging to establish causal relations between risk factors and outcomes. At present identification of a specific time for dementia onset is challenging, and while it may partly be possible with the available biomarkers, these biomarkers are expensive, time-consuming and invasive, limiting the use in the general population. Secondly, most epidemiological studies are conducted in older populations with shorter follow-up times, and there are few large prospective long-term studies starting in midlife or at younger ages where the preclinical neuropathological changes are less likely to have started [69]. Lastly, although we now know much about individual risk factors for dementia, we do not know how they interact or which risk factors account for what proportion of dementia cases. These issues inherent to dementia justify the need for further studies on potential risk factors, their potential interactions and their temporal relationship to dementia and cognitive impairment, especially in larger prospective studies with longer follow-up.

1.4 Prevention and treatment

1.4.1 Primary, Secondary and Tertiary Prevention

Prevention is divided in three levels: primary, secondary and tertiary prevention. The ultimate goal with primary prevention is to prevent the onset of specific diseases. The main focus is risk reduction by preventing exposures to certain hazards. This is done e.g. through education and health promotion initiatives. Secondary prevention involves initiatives to detect and treat preclinical pathological changes. The goal is to control disease progression, postpone onset of clinical symptoms resulting in reduced incidence of manifest impairment, and a compression of morbidity. Finally, tertiary prevention seeks to minimize the damage caused by the disease, its recurrence and related disabilities. The main interventions are treatment and rehabilitation aiming to enhance the ability to function, increase quality of life and/or prolong life expectancy [70]. Dementia and AD treatment has traditionally aimed at tertiary prevention, however during the past decade(s) the focus has moved to secondary and even primary prevention [71]. The perceived reason is the fact that several clinical trials of potential disease modifying interventions have failed to meet their primary outcomes in study populations already diagnosed with AD dementia. The purpose of interventions across the levels of prevention in relation to stage of dementia is outlined in figure 5.

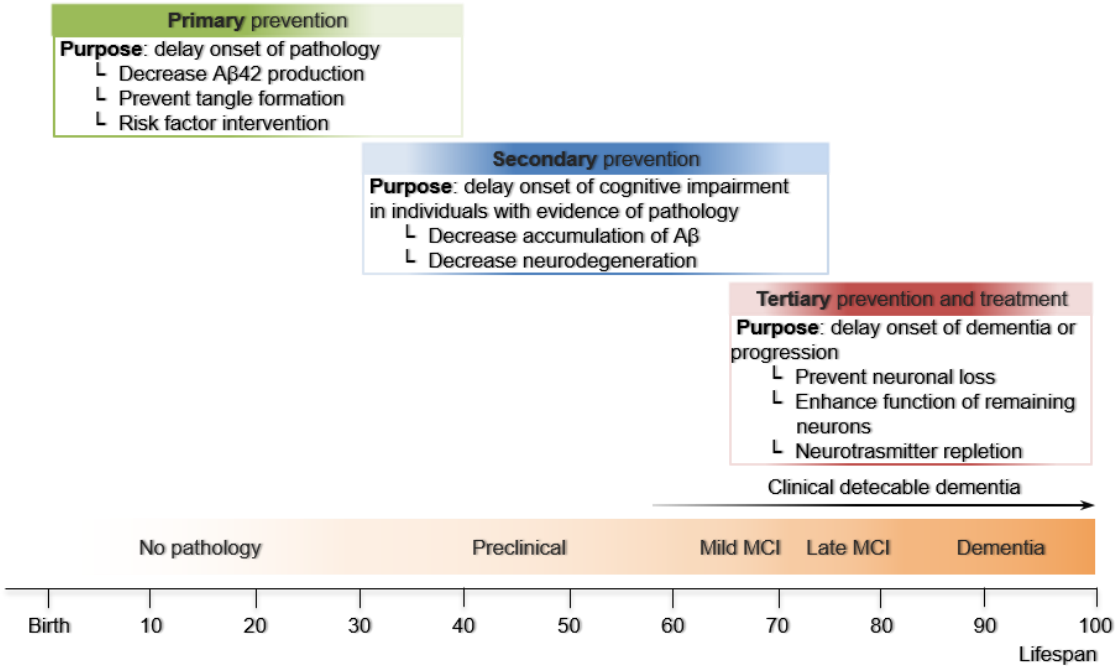

Figure 5: Overview of primary, secondary and tertiary prevention as a function of dementia disease stage. Figure made with inspiration from [72].

Several prevention trials are on-going including both pharmacological and non-pharmacological interventions. The pharmacological approaches include early intervention studies for both EOAD and LOAD. The Dominantly Inherited Alzheimer Network Trial (NCT01760005) will assess the potential disease modifying effect of gantenerumab and solanezumab, two monoclonal antibodies targeting Aβ, in individuals with genetic mutations leading to EOAD. LOAD studies include the Anti-Amyloid Treatment in Asymptomatic Alzheimer's study (A4 study, NCT02008357). This study will assess the effect of solanezumab in asymptomatic or very mildly symptomatic individuals who have biomarker evidence of Aβ deposition. The A4 study will be followed by other public-private partnership trials; the A5 and the A3 studies. The A5 trial (NCT02569398) will test a β-secretase cleaving enzyme (BACE) inhibitor in a similar preclinical population as the A4 study, while the so-called Ante-Amyloid Prevention of Alzheimer's disease study (A3) is planned to identify individuals with a subthreshold level of Aβ who are at high risk for further accumulation [73]. The TOMMORROW trial (NCT01931566) will assess the utility of a genetic-based biomarker risk assignment algorithm and the effect of the insulin sensitizer pioglitazone, in an asymptomatic high risk population carrying the *APOE* and *TOMM40* risk genes [74].

Recent population-based randomized controlled trials (RCTs) assessing the impact of multi-domain lifestyle interventions on cognitive decline or incident dementia include the FINGER, MAPT and PreDIVA studies [75–77].

1.4.1.1 Potential challenges

Some challenges are still in the way of successful prevention. First, as AD and other dementia disorders are multifactorial conditions with several different pathways driving the pathogenesis, each of these pathways might need different interventions, or likely a combination of different interventions. Therefore, it is of utmost importance to identify who to treat with a certain treatment and also a matter of when we should intervene. Since the “window of opportunity” is believed to be years before dementia onset where the cognitive decline is normally limited, we need to identify the subjects with highest likelihood of progression and develop sensitive outcome measures including cognitive tests and reliable biomarkers to measure efficacy.

Finally, in the absence of disease modifying interventions, ethical concerns are raised regarding the disclosure of an elevated risk e.g. through genetic testing or biomarker positivity.

1.4.2 Current and Future Treatment Options

The current available treatments for dementia are limited to symptomatic relief. In Denmark, three cholinesterase inhibitors (donepezil, rivastigmine and galantamine) are approved for treatment of mild to moderate AD and memantine, a non-competitive N-methyl-d-aspartate acid (NMDA) receptor antagonist, for treatment of moderate to severe AD [78]. The cholinesterase inhibitors postpone the worsening of symptoms for an average of up to 12 months, but only for about half of the treated subjects [79]. Cholinesterase inhibitors have shown no clinical effect in subjects with MCI [80].

The central role of Aβ in the pathogenesis of AD has made this the prevailing drug target in the development of disease modifying interventions. Other relevant treatment strategies target tau or alternative pathways that have emerged because of the multifactorial and heterogeneous nature of LOAD. The strategies are outlined in table 2.

Table 2: Drug targets in the development of disease modifying interventions for AD. Aβ: Amyloid-β. The table include information from [81–88].

Target	Strategy	Rational
Aβ	Reduce production	Stimulation of α-secretase favours the non-amyloidogenic metabolism of APP reducing formation of $A\beta_{1-42}$. Inhibition of β- or γ-secretase reduces the level of toxic Aβ species.
	Enhance clearance	Direct activation of the Aβ-degrading enzymes or blocking the inhibitor of a protease that is required to activate an Aβ-degrading enzyme. Antibody-mediated amyloid clearance by stimulating an immune response against monomeric-, oligomeric- or fibrillar species of Aβ.
	Prevent aggregation	Monomeric Aβ molecules can form oligomeric aggregates that are thought to initiate the pathogenic cascade.
Tau	Stabilization of microtubule	Does not interfere directly with tau, the rational is rather to compensate for the loss function once tau dissociates from the microtubules.
	Modulation of phosphorylation	Hyperphosphorylation is critical for tau to detach from microtubules and is believed to be a prerequisite for it to aggregate.
	Prevent aggregation	The initial stages of the aggregation process are the best stage to inhibit aggregation as the oligomers are considered the toxic specie.
	Enhance clearance	Stimulate an immune response against pathologically modified forms of tau and thereby enhance the clearance of tau.
Other	Insulin sensitizer	Antagonism of the peroxisome-proliferator activated receptor γ (PPARγ) increases Aβ phagocytosis and thereby the clearance of Aβ.
	Anti-inflammatory	Combination therapy consisting a mast cell stabilizer and a non-steroid anti-inflammatory agent inducing an anti-inflammatory response. Inhibition of the receptor for advanced glycation end products as it induces inflammation and oxidative damage.

1.4.3 Precision Medicine

The introduction of the term “precision medicine” marked the end of the traditional “one size fits all” approach and kick-started the paradigm shift towards individualized medicine [89]. The National Institute of Health in the United States defines precision medicine as: "an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person" [90] and with the new initiative on precision medicine outlined by Barack Obama in early 2015, efforts are now focused to accelerate progress within this new era [91].

In the dementia field, the concept of precision medicine is also emerging and is considered the key to success in the development of disease modifying interventions. Researchers are now recognizing that the complex multifactorial nature of LOAD will require a comprehensive exploratory systemic approach to understand the complex mix of processes that underlie the pathogenesis [92,93]. Three key elements to precision medicine in AD have been outlined: *i)*

comprehensive risk assessment, *ii)* tools for early detection of pathophysiological processes and *iii)* "customizing" interventions based on an individual's molecular drivers [92]. A more detailed understanding of the pathogenesis of dementia and AD is however still needed to pave the way for precision medicine. Unravelling the various pathological pathways and their interactions is key to determine the risk of developing AD on an individual level [94]. A comprehensive risk assessment is necessary for prognosis and early intervention and should facilitate the identification of individuals with high risk for incident dementia, but also identify individuals with preclinical pathological evidence with a high likelihood of progression. The individual's molecular drivers should aid in identification of optimal treatment with an optimal risk-benefit profile [95].

Biomarkers are an integrated part of precision medicine and are therefore considered key to unlocking precision medicine [96]. In AD, a range of well-established biomarkers exists, however their widespread use at the population level is limited, as outlined in section 1.7.

1.5 Pathways to dementia: The multifactorial trigger and hit hypothesis

The pathways leading to FTD and DLB are still largely unknown and therefore this section is limited to pathways leading to AD. Beside synaptic dysfunction, extracellular deposition of Aβ plaques and formation of intracellular neurofibrillary tangles (NFT), there are several other processes implicated in the pathology including structural and functional abnormalities of the mitochondria, chronic inflammation and oxidative damage [97]. The mitochondrial abnormalities occur early in AD pathology and can promote both synaptic damage and apoptosis [98]. Oxidative damage is speculated to have a causative role in the pathogenesis of AD since it has been shown to occur prior to Aβ plaque formation [99]. The high frequency of coexisting pathologies from autopsy confirmed human studies indicates that the various pathological pathways are interconnected. This is further supported by findings from animal studies where it has been shown that tau is required for Aβ to impair synaptic plasticity in mice hippocampus and that tau deletion in mice affects intracellular $A\beta_{1\text{-}42}$ clearance resulting in extracellular plaques [100,101]. It is therefore reasonable to think that several pathways will lead to AD, and that each of these pathways are somewhat heterogeneous (as outlined in figure 6). Whether there is a common upstream driver for all pathways and how interactions between genetic and environmental factors are driving the disease progression is still not fully understood. The evidence, that Aβ plaques are required but insufficient to independently drive the pathological conversion of tau, suggests that other factors (hits) are necessary to drive the pathological progression. Thus, there is still a range of unanswered questions: what is the initial trigger(s)? and how many subsequent hits are needed for the disease to manifest clinically? The multifactorial model outlined in figure 6 could explain why many of the reported genetic and environmental risk factors are neither sufficient nor necessary for the disease to occur and could also suggest that different pathways may need

different interventions and if several pathways are interacting several co-administered pharmacological interventions are probably needed.

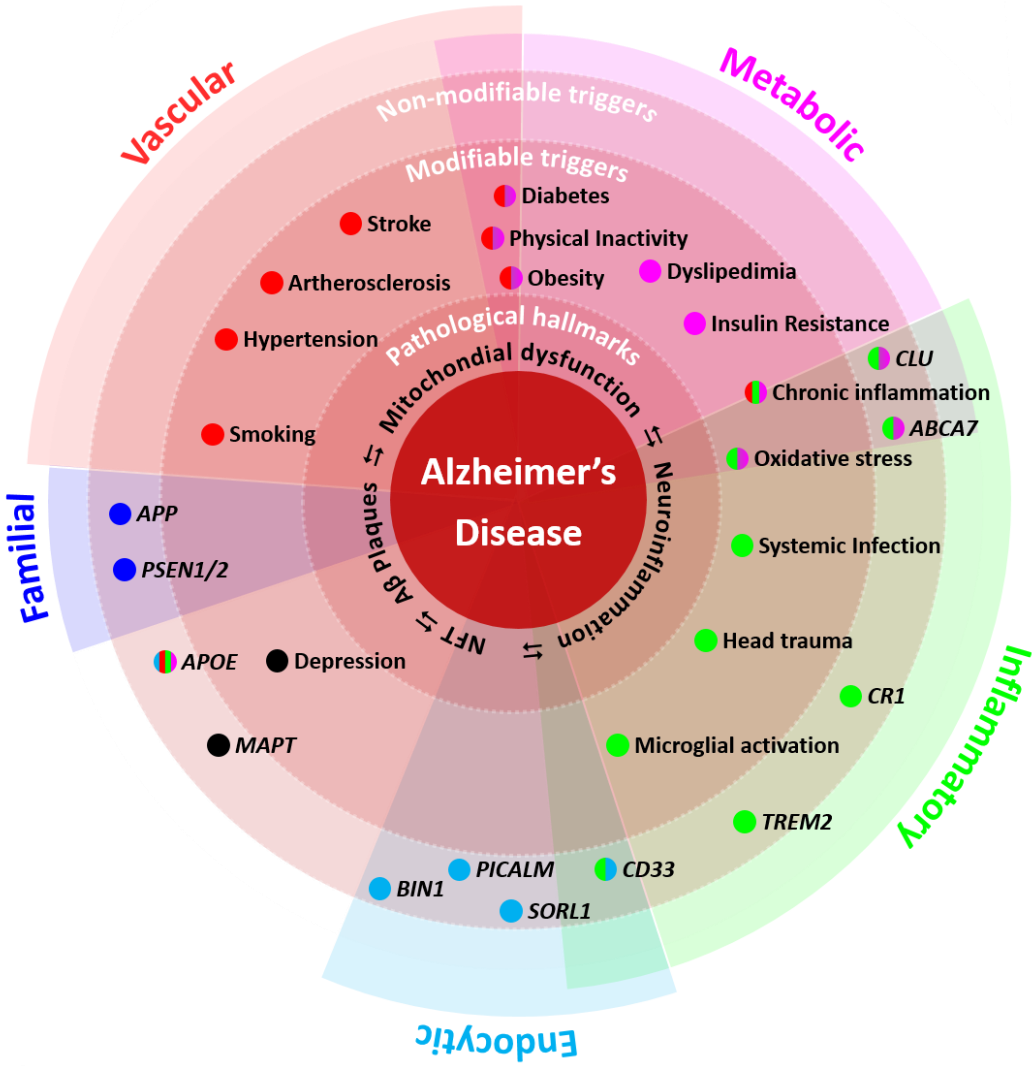

Figure 6: Hypothetical pathways to Alzheimer's disease including genetic and environmental triggers. NFT: Neurofibrillary tangles, Aβ: Amyloid-β. Red color represent a vascular pathway, magenta is a metabolic pathway, green represent an inflammatory pathway, and the endocytic pathway is cyan while the familial and dominantly inherited pathway is blue.

1.6 Neuropathology

The common denominator of all neurodegenerative dementias is that cognitive function is impaired due to synapse degeneration and neuronal death. Several hypotheses have been suggested for the etiology of the main dementia subtypes. However, what triggers the characteristic hallmarks like the Aβ plaques, NFT and Lewy Bodies, and the sequence of events driving the disease progression is still not fully elucidated.

The molecular defects observed in EOAD gave rise to the “amyloid hypothesis” which still is the dominant hypothesis for AD, and since EOAD and LOAD share many clinical and histopathological features this hypothesis was also adopted to LOAD [102]. It was originally proposed as one pathway with a specific temporal relation where failure in clearance and overproduction of the Aβ peptide drives the downstream events including hyperphosphorylation of tau. During the past 25 years, the hypothesis has been challenged on several occasions, especially in light of the recent anti-amyloid trials that did not provide any clinical improvement in relation to AD [103,104]. Inflammatory involvement through microglial activation triggered by certain damage signals, e.g. Aβ, was later proposed in the “revitalized tau hypothesis” [105]. The pathways linking Aβ and tau to synapse degeneration and neuronal death are however still largely unknown, and whether the hallmarks share the same pathway, belong to independent pathways or are dual pathways that interact to exacerbate one another are still debated [106–108]. In a broad sense VaD is caused by problems in the supply of blood to the brain. It affects the neural networks and arises from systemic, cardiac, and local large and small vessel disease. It may manifest as a result of a single infarct, multiple infarcts, or microvascular insults [109].

A striking similarity between all neurodegenerative diseases is the accumulation of misfolded protein aggregates and deposition of aggregates in so called inclusion bodies in different areas of the brain. As a consequence of post-mortem findings, the fibrillated end-products have traditionally been considered the toxic species. However, certain more recent evidence suggests that it may be the soluble forms of the proteins that are responsible for the neurotoxicity [110–112].

1.6.1 The proteopathy of neurodegenerative diseases

As illustrated in table 3 there is a marked overlap between the proteins involved in the different dementia-related neurodegenerative diseases. Neither Aβ plaques nor NFTs are specific for AD, they also accumulate in other neurodegenerative diseases. Aβ accumulate in cerebral amyloid angiopathy while NFTs are formed in other tauopathies like FTD [113,114]. Furthermore, Aβ plaques, NFT and Lewy bodies are found in cognitively normal elderly people, suggesting that these characteristic hallmarks are not in themselves sufficient to cause dementia [115–117]. The Aβ plaques were shown to consist of the peptide Aβ that aggregate and accumulate in the extracellular space [118]. In 1986, the microtubule associated protein tau was identified as the main constituent of the NFTs [119]. Lewy bodies are spherical clusters within the cerebral cells consisting of proteins α-Synuclein and ubiquitin entangled with abnormally phosphorylated neurofilament protein [120]. Tau has also been found to co-localize with α-Synuclein in Lewy Bodies [121].

Table 3: Overview of major aggregating proteins in neurodegenerative diseases. Aβ: Amyloid-β; FUS: Fused in Sarcoma protein; TDP-43: TAR DNA-binding protein 43; AD: Alzheimer's disease; VaD: Vascular dementia; FTD: Frontotemporal dementia; DLB: Dementia with Lewy Bodies.

Aggregating protein	**Proteopathy**
Aβ [122]	AD, cerebral amyloid angiopathy, DLB
Tau [123–125]	AD, VaD, FTD, corticobasal degeneration, progressive supranuclear palsy, chronic traumatic encephalopathy, Pick's disease
Prion protein [126]	Creutzfeldt-Jakob Disease
α-Synuclein [127,128]	AD, Parkinson's disease, DLB, multiple system atrophy
TDP-43 [129,130]	AD, FTD, amyotrophic lateral sclerosis, chronic traumatic encephalopathy
FUS [129,130]	FTD, amyotrophic lateral sclerosis
Huntingtin [131]	Huntington's disease

1.6.2 Post-translational modifications implicated in neurodegeneration

Post-translational modifications (PTMs) of the different proteins are known to play a central regulatory role in both physiological and pathological processes in a range of age-related non-communicable diseases, including neurodegeneration [132]. Since the PTMs arise from specific combinations of covalent modifications e.g. enzymatic processing and proteins they are considered the defining feature of the molecular pathology and therefore they have the advantage over intact proteins to create a specific profile of each of the neurodegenerative disorders [133]. Several PTMs are enriched within the various inclusion bodies and are found to exist at higher levels in the brains of subjects suffering from neurodegenerative diseases, suggesting that certain modified species of these proteins might be more relevant biomarkers than the full-length protein [134,135]. The tau protein is involved in a range of neurodegenerative disorders known as tauopathies. In the following section focus will be on the abnormal processing of tau involved in the pathological self-aggregating process.

1.6.2.1 The processing of Tau

PTMs initiate the pathological processing cascade of tau. One of the earliest modification of tau is phosphorylation. The hyperphosphorylation occurs at several sites and results in a dissociation of tau from the microtubules [136,137]. Another early PTM is caspase cleavage leading to a truncation of the protein [123,135]. The PTMs increase the susceptibility of tau to self-aggregate. In the process of aggregation, tau monomers polymerize to form toxic oligomers, which assemble into paired helical filaments that eventually form NFTs [138]. Evidence indicates that there is a complex interaction between phosphorylation and truncation of tau and a recent model proposes that early truncation at the C-terminal generates neurotoxic tau species while phosphorylation may have a neuroprotective role [139]. Several of the PTMs are largely preserved across the different tauopathies, including AD [140]. The tau protein is also known to undergo other PTMs

such as glycosylation, nitration, ubiquitination and oxidation, however the role of these modifications is less well understood [141].

1.6.2.2 Caspase cleavage of tau

Truncation of tau is known to accelerate the aggregation of tau in vitro [123,135]. Several caspase-cleavage sites have been identified and both caspase-3 and caspase-6 were found to cleave tau in the AD brain [142]. Interestingly, the N- and C-terminal regions of tau inhibit the polymerization of tau in vitro, implying that caspase cleavage is indeed an important early promotor of the aggregation process [143,144]. Among the known cleavage sites, an initial cleavage has been found to occur specifically at Asp421, catalysed preferably by caspase-3. This cleavage site is well-validated both in vitro and in vivo. Importantly the cleavage fragment (named ΔTau or Tau-C) is associated with clinical severity of dementia and neuropathogical severity [145]. Caspase cleavage was also proposed as a mechanistic link between Aβ and NFTs since Aβ was found to induce apoptosis and thereby the activation of caspases in vitro [146]. A novel pathogenic process related to caspase-2 cleavage of tau at Asp314 was recently published. Unlike the other PTMs described above, this truncation is unique in the sense that it resists fibrillation. The fragment was shown to drive other tau species to the dendritic spines, leading to reduced excitatory synaptic transmission and induced memory deficits in mice [147].

1.7 Biomarkers of Neurodegeneration

A biomarker is defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” [148]. Biomarkers have a broad range of use in research, clinical practice and drug development and are classified per their application. The “BIPED” (Burden of Disease, Investigative, Prognostic, Efficacy of Intervention and Diagnostic) classification system as outlined in table 4 was originally designed for osteoarthritis but has also been proposed to be used for classification of biomarkers in the AD field [132,149].

Table 4: The “BIPED” (Burden of Disease, Investigative, Prognostic, Efficacy of Intervention and Diagnostic) biomarker classification system. Modified from [149,150]

	Biomarker Categories (BIPED)				
	Burden of disease	**I**nvestigative*	**P**rognosis	**E**fficacy (**P**redictive)	**D**iagnostic
Predictor of	Disease Activity, Stage / Severity	Explorative use	Predict onset, Progression of disease	Monitor treatment Predict efficacy	Classify individuals as either having or not having the disease
Design	Cross-sectional	NA	Longitudinal	Longitudinal	Cross-sectional
Ideal Properties	b > e > a > d	NA	d > a > b > e	e > b > a > d	c > a > b > d > e

* Remain to be included in one of the other BIPED categories due to insufficient clinical evaluation. a: high sensitivity, b: reliable specificity, c: detectable early in the disease course, d: inexpensive, easy accessible and non-invasive, e: repeated measurement feasible. NA: Not applicable

Diagnostic biomarkers should aid in the distinction between diagnostic groups e.g. diseased versus healthy controls at a single point in time. Longitudinal biomarkers include prognostic and predictive biomarkers. A prognostic biomarker is used to obtain information about a future outcome e.g. progression of a disease or death, while a predictive biomarker should identify those subjects that are likely to respond to a given therapy. It is possible that a single biomarker can have several applications [151,152].

Established biomarkers of neurodegeneration belong to two categories; CSF biomarkers and neuroimaging biomarkers (see section 1.7.1). These biomarkers aid in the diagnosis of dementia-related diseases, however their large-scale use in clinical practice is limited because they are measured using expensive, time-consuming and invasive procedures (the lumbar puncture). In 2010, a hypothetical model of the major biomarkers describing the temporal evolution across the clinical disease stages of AD was put forward [153]. The model, which was updated in 2013 [154], include the most well-established biomarkers assessing Aβ deposition and neurodegeneration (figure 7).

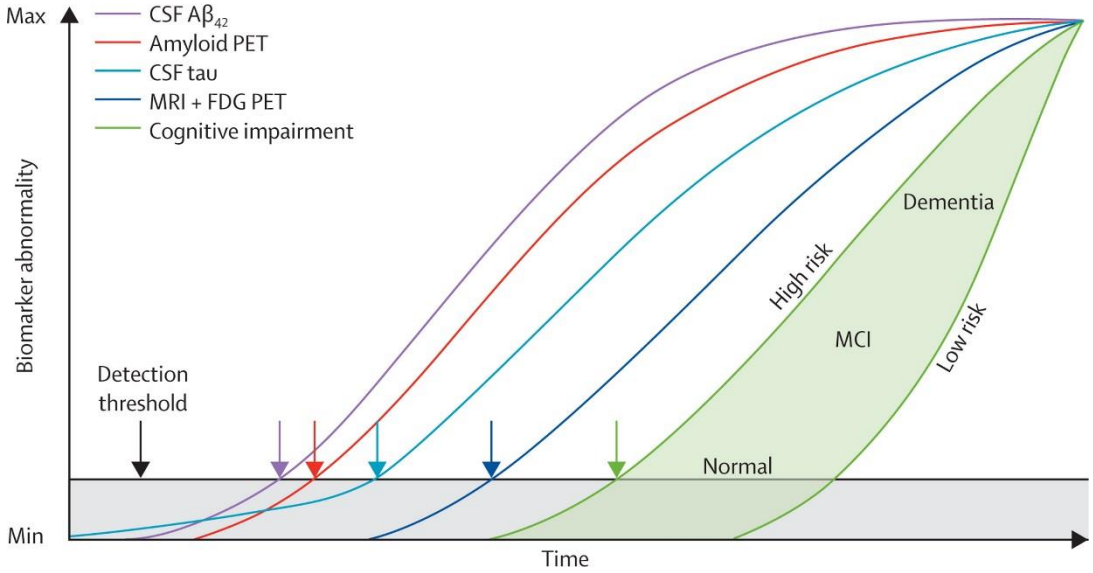

Figure 7: The updated hypothetical model of dynamic biomarkers of the Alzheimer's disease pathological cascade. FDG: Fluorodeoxyglucose; MCI: Mild Cognitive Impairment; PET: Positron Emission Tomography; MRI: Magnetic Resonance Imaging; Figure adapted from [154]

An important update in the revised model was that CSF tau may become abnormal before Aβ as illustrated in the lower left of figure 7. The abnormality can however not be detected with the current sensitivity of the analytical methods. This evidence was based on autopsy data published by Braak and Del Tredici [155], and later also confirmed by Young and colleagues in a subpopulation using a data-driven modelling approach of CSF biomarker data from the Alzheimer's Disease Neuroimaging Initiative [156].

1.7.1 Cerebrospinal Fluid and Imaging Biomarkers

The three core CSF biomarkers: $A\beta_{1-42}$, t-tau and p-tau are used as diagnostic biomarkers for AD. $A\beta_{1-42}$ is found in lower concentration in subjects with AD while t-tau and p-tau are found to be elevated in diseased subjects [157]. The diagnostic accuracy of these biomarkers has been assessed in a range of subjects and in 2011 this resulted in an incorporation of these biomarkers into the diagnostic research criteria for preclinical AD [11]. Beside the well-established diagnostic utility at both the MCI and dementia stages there is also evidence supporting a prognostic potential, as it was shown that the markers can predict a poor long-term clinical prognosis from an early disease stage [158]. There are also other emerging CSF biomarkers that have shown promising results however these will not be discussed in this thesis.

There are several well-established imaging techniques used in the diagnosis and prognosis of AD and other types of dementia. Brain atrophy measured with MRI can aid in the diagnosis of dementia but more promising hippocampal atrophy has been shown to predict the conversion from MCI to dementia making it useful for early identification [159]. Fluorodeoxyglucose (FDG) uptake is a marker of synaptic activity and neuronal density. FDG PET is used to assess neuronal injury and dysfunction and was found to predict longitudinal cognitive decline suggesting that this marker may be useful in the selection of patients for clinical trials [160]. PET tracers visualizing the key pathological hallmarks of AD, Aβ plaques and NFT, have also been developed. Amyloid PET is becoming widely adopted and has been incorporated as a selection criterion for several of the on-going and planned prevention trials [73]. Tau PET is emerging but due to problems with selectivity and off-target binding, it is still lagging behind Amyloid PET [161].

1.7.2 Blood-based Biomarkers

Blood as a source for biomarkers hold promise since it persists many of the characteristics that define an optimal marker. Unlike CSF, it can be obtained rapidly by a minimally invasive and inexpensive procedure, and it allows for repeated measures over time [152]. The success has however been highly limited due to a lack of understanding of how a peripheral biomarker signal relates to processes occurring centrally in the brain. Another challenge is that the blood-brain-barrier (BBB) exerts a natural limit for the transfer of substances from the central nervous system (CNS) to the peripheral blood. The transport of substances from the CNS into the periphery may be strictly controlled under normal conditions, however pathological alterations can potentially trigger a release of brain specific proteins into the periphery [162] or on the other hand interfere with the clearance of potentially harmful substances from the brain [163]. The disruption of the BBB may be phenotypically determined to the extent that the degree of BBB involvement is very heterogeneous across the diverse etiologies of AD [163]. Beside the transport over the BBB two recently described clearance systems were identified in the brain; a glymphatic system [164] and

a lymphatic system [165] proving that macromolecules or fragments of these can be released from the brain and transported to the peripheral system.

Several different approaches have been used for the identification of blood-based biomarkers. The approaches generally fall into two categories; *i)* the *omics* approaches including genomics, proteomics, metabolomics and transcriptomics searching for patient-specific multi-biomarker profiles and *ii)* single protein candidates or fragments hereof [166]. The former will not be discussed further in this thesis. Among the most extensively studied single protein candidates are Aβ and tau.

As outlined earlier, the processing of both Aβ and tau involve several PTMs and they appear to be key pathological events. Specifically focusing on protein fragments generated by disease-specific combinations of proteins and proteases could theoretically result in an easier release from the brain, as the fragments due to their smaller size can pass through the BBB more easily, and at the same time be more specific for pathological changes than their intact counterparts [133,166]. The resulting protein fragments are referred to as neo-epitopes. The use of neo-epitope biomarkers in the AD field is not new, as both Aβ and p-tau are examples of neo-epitope CSF biomarkers generated by cleavage and phosphorylation, respectively.

This thesis focus on the utility of two such neo-epitope fragments of tau, named Tau-A and Tau-C. Emphasis will not be put on the technical aspects of the biomarker development and measurement, but solely explore the potential use of these markers in a population-based cohort.

1.7.2.1 Tau Neo-Epitope Biomarkers in Serum

Processing of tau in the context of biomarker development is of great interest as the initial proteolytic cleavage appears to catalyse the aggregation cascade. As previously mentioned, the Tau-C fragment is generated by the caspase-3-mediated cleavage at Asp421, and its association with the AD pathology is well-established. The second fragment, Tau-A, is an N-terminally truncated fragment of tau generated by ADAM-10-mediated cleavage at Ala152. The development of this fragment as a potential biomarker for AD is based on the hypothesis that tau is exposed to secretase-mediated cleavage either directly in the brain or as a subsequent processing in the periphery [167]. Both potential biomarkers have previously been tested in minor clinical settings. In the initial evaluation of Tau-A, the marker was found to have an inverse correlation with cognitive function in a cross-sectional study of subjects with a clinical diagnosis of probable AD [167]. In serum samples from a Phase III clinical trial of subjects with mild to moderate AD, Tau-A and Tau-C showed very limited diagnostic utility, but the ratio between Tau-A and Tau-C was related to the rate of disease progression [168]. In relation to sports-related concussion the level of Tau-A was found to peak rapidly after an injury, and within a timeframe of

12 hours the marker could identify the subjects with complicated concussion, suggesting that Tau-A is indeed related to the extent of injury in the brain [169]. The potential for differential diagnosis was assessed in an observational cross-sectional study of patients originally presenting with memory complaints. The fragments, Tau-A in particular, were found to be elevated in patients with MCI and mild-to-moderate AD when compared to patients with other dementias and patients with non-dementia-related memory complaints [170].

1.7.2.2 The Need for Non-Invasive Biomarkers in Dementia

Despite the fact, that the existing CSF and imaging biomarker for AD and dementia are highly accurate, barriers to clinical implementation exist as the invasiveness and high expenses of these procedures, preclude large-scale use at the population level. The blood based biomarkers are therefore key for early identification and prognosis, as a well-validated blood-based biomarker may be used as a screening tools to identify the subjects with high disease risk, or subjects with a high likelihood of progression. Additionally, sampling at multiple time-points is another key feature of blood-based biomarkers useful not only as an indicator of disease onset or risk, but also to carefully monitor treatment efficacy.

2

Aims and Study Design

2. Aims and Study Design

Given the increase in average life expectancy and the subsequent rise of dementia prevalence, the identification of subjects at high risk of developing dementia is key for prognosis and early intervention. In their present form, no single diagnostic instrument or combination of instruments is sufficiently developed to be used for dementia screening.

2.1 Research Questions and Hypothesis

1) What risk factors are associated with dementia and cognitive dysfunction in late-life?
2) Are blood levels of tau neo-epitope biomarkers associated with dementia, and if so, can these be used as prognostic biomarkers?

The research questions lead to the following **hypothesis**:
Modifiable risk factors and blood-based biomarkers are relevant predictors of dementia and can be used as first step in a multi-stage screening process for the identification of subjects in most need on preventive interventions or to identify subjects suitable for enrollment in clinical trials.

2.2 Aims

The **overall aim** was to obtain a better understanding of the underlying comorbidities driving the pathogenesis for the clinical representation of dementia in elderly women. The overall aim led to the following **specific aims**:

1. Identify risk factors for incident dementia and its subtypes (**Paper II**).
2. Assess the association between precursors of type 2 diabetes and cognitive dysfunction. (**Paper III**).
3. Assess the risk of progression to dementia in subpopulations of women with signs of cognitive deficits and investigate cognitive courses in late-life including an identification of risk factors specifically associated with the progression (**Paper IV**).
4. Evaluate the possible utility of two novel serological biomarkers of truncated tau as predictors of incident dementia in women **(Paper V**).

The Prospective Epidemiological Risk Factor Study (PERF), a population-based prospective cohort study of elderly women in Denmark founded the basis of the current work. A cohort profile with a detailed outline of the overall study design, aims, available data and key findings was published recently (**Paper I**).

2.3 Study Design

The flow chart depicted in figure 8 outlines a short summary of the study designs including size of study populations, endpoints, primary exposures of interest, methods of follow-up and the statistical methods used in each of the included papers.

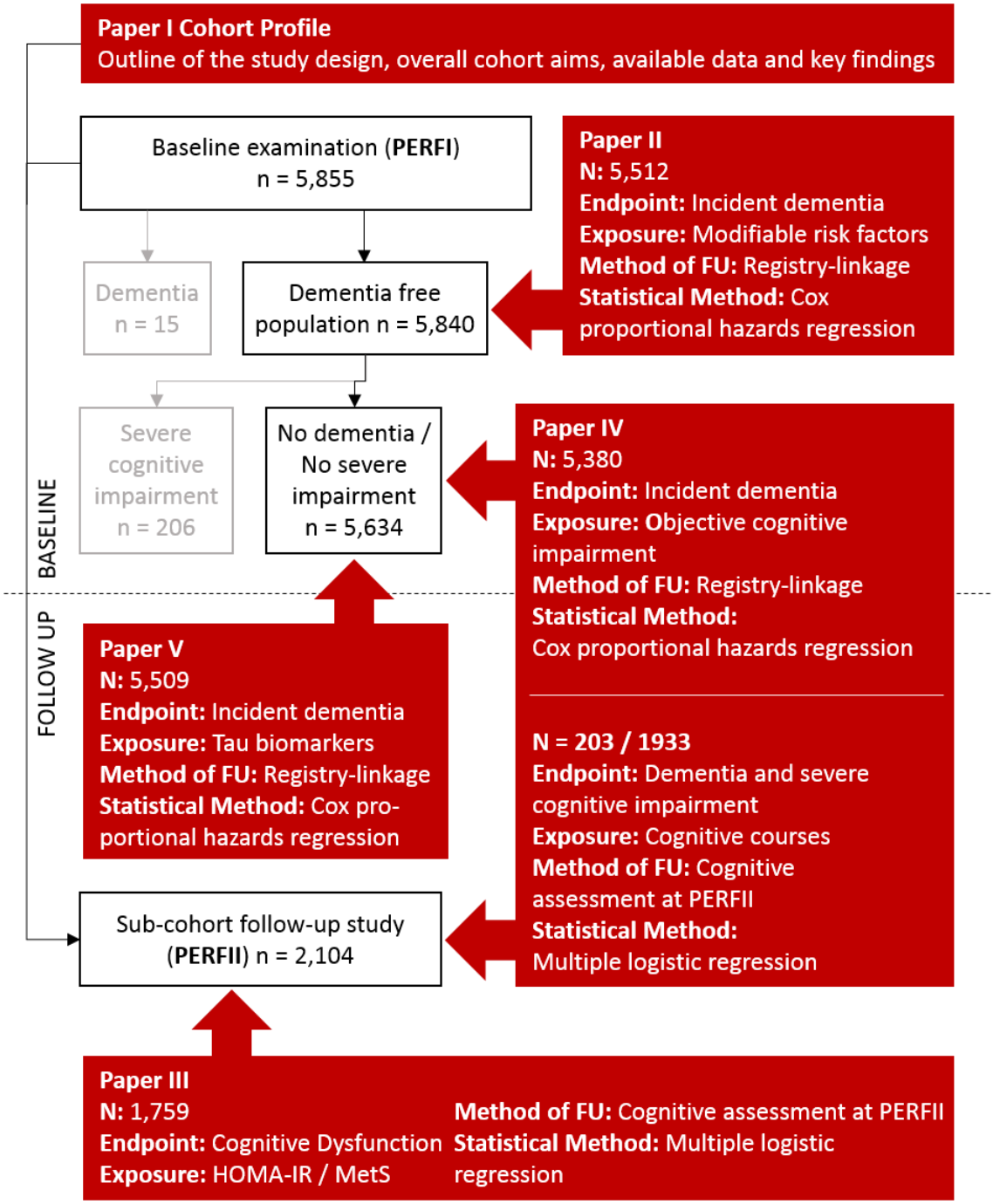

Figure 8: Flow chart depicting the relation between the individual papers and the baseline and follow-up studies. Study population size, primary endpoint and exposure of interest, method(s) of follow-up and the statistical method used are listed for each paper. FU: Follow-up.

3

The Prospective Epidemiological Risk Factor (PERF) Study

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Cohort Profile

Cohort Profile

Cohort Profile: The Prospective Epidemiological Risk Factor (PERF) study

J.S. Neergaard,[1]*† K. Dragsbæk,[1†] S.N. Kehlet,[1] H.B. Hansen,[1] G. Hansen,[1] I. Byrjalsen,[1] P. Alexandersen,[2] L.M. Lindgren,[3] A.R. Bihlet,[1] B.J. Riis,[1] J.R. Andersen,[1] P. Qvist,[1] M.A. Karsdal[1]and C. Christiansen[1]

[1]Nordic Bioscience A/S, Herlev, Denmark, [2]Center for Clinical and Basic Research, Vejle, Denmark and [3]Center for Clinical and Basic Research, Ballerup, Denmark

*Corresponding author. Nordic Bioscience A/S, DK-2730 Herlev, Denmark. E-mail: jsn@nordicbio.com
†These authors contributed equally to this work.

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Why was the cohort set up?

The world's population is ageing.[1] In Europe alone, the elderly population over age 65 will double from 88 to 153 million and the fastest growing segment of the population will be those over 80, tripling in number from 24 to 60 million in 2060.[2] Low birth rates and increasing longevity are the key factors in this shifting trend in ageing demographics.[3] Maintaining a healthy life is important, as an ageing population in good health will limit the pressure on health care systems.[3,4] However, it is likely that risk factors compromising healthy ageing, such as smoking, obesity, excess alcohol consumption, unemployment, and lack of physical activity, will negatively affect the years people spend in good non-treatment requiring health.[1,5] In 2006, it was estimated that women in the Western European countries are expected to live about 80% of their lives in good health. In other words, this predicts a healthy life expectancy up to 20% shorter than the total life expectancy.[4] Focus on a healthy elderly population is therefore of greater interest than ever.

Age-related diseases are usually expressed as chronic conditions commonly occurring in combination with each other, with cardiovascular disease and type 2 diabetes being two of the most common age-related diseases in the EU.[1,4] The ability to understand the links and underlying pathogenesis are therefore crucial in order to be able to shift the treatment regimen from disease treatment to preventive measures, thereby prolonging the period that elderly people spend in good health.

The Prospective Epidemiological Risk Factor (PERF) Study, an observational, prospective cohort study of Danish postmenopausal women, was designed with the purpose of obtaining a better understanding of the development of age-related diseases in postmenopausal women. In 1999, the source population was identified from a database of subjects who had previously been screened for participation in one of 21 clinical randomized controlled trials (source studies[6–24]). All living subjects with a unique personal subject identification number and a valid postal address constituted the source population (a total of 8875 women). The source studies were all initiated with the purpose of obtaining further knowledge about the aetiology and pathogenesis of menopause-related diseases, and included both intervention and non-intervention studies (as illustrated in Figure 1). The source population therefore consists of women who previously participated in a source study or were screened, without being randomized. The first source study was initiated in 1977. In 1999, the first participants were enrolled in the epidemiological cohort of the PERF study (henceforth termed PERF I), and from September 2013 to December 2014 the participants completed the latest follow-up (termed PERF II). The total number of participants attending the baseline examination

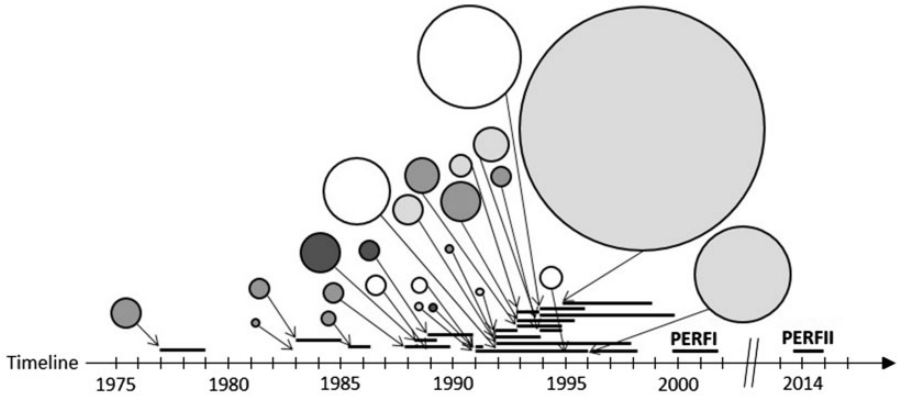

Figure 1. Source studies defining the source population for the Prospective Epidemiological Risk Factor (PERF) study illustrated on a timeline including number of participants, study duration and type of intervention. Bubble size is proportional and equivalent to the number of participants. All bubbles are sized relative to the largest study (N = 2,789). Color represents type of intervention; white bubbles are nonintervention studies, light grey bubbles are placebo controlled bisphosphonate studies, medium grey bubbles are placebo controlled hormone replacement therapy studies and dark grey bubbles represent other types of intervention studies. Black lines correspond to the study duration (in years).

(PERF I) was 5855, of whom 2103 attended the follow-up visit (PERF II) approximately 15 years later. Including the source studies, the study may be considered an ambidirectional cohort study with a total observation period of more than 35 years. The PERF I and PERF II studies were funded by the Danish Research Foundation (Den Danske Forskningsfond).

The current paper outlines the study design, the study population and an overview of the collected data together with a summary of the key findings until now.

Who is in the cohort?

Inclusion

In 1999, an invitation to attend the baseline examination was sent to the entire source population ($n = 8875$) except for those who died since their last contact with the clinic ($n = 732$). In this subgroup, causes and times of death were collected from the Danish National Death registry. No active recruitment initiatives besides the invitation was taken, leaving a total of 5855 (72%) women to consent and attend the baseline examination of the epidemiological PERF I study conducted at the Center for Clinical and Basic Research (CCBR) in cities of either Aalborg or Ballerup, Denmark, between 1999 and 2001. There were no in-/exclusion criteria at the time of enrolment in the cohort study.

A subcohort (PERF II), initially being enrolled at the CCBR clinic in Ballerup, was re-investigated in 2013-14, when invitations were sent to 2813 women from the original PERF I cohort. Those subjects who did not respond to the written invitation were contacted by phone. As a result of this active recruitment, a total of 2103 (75%)

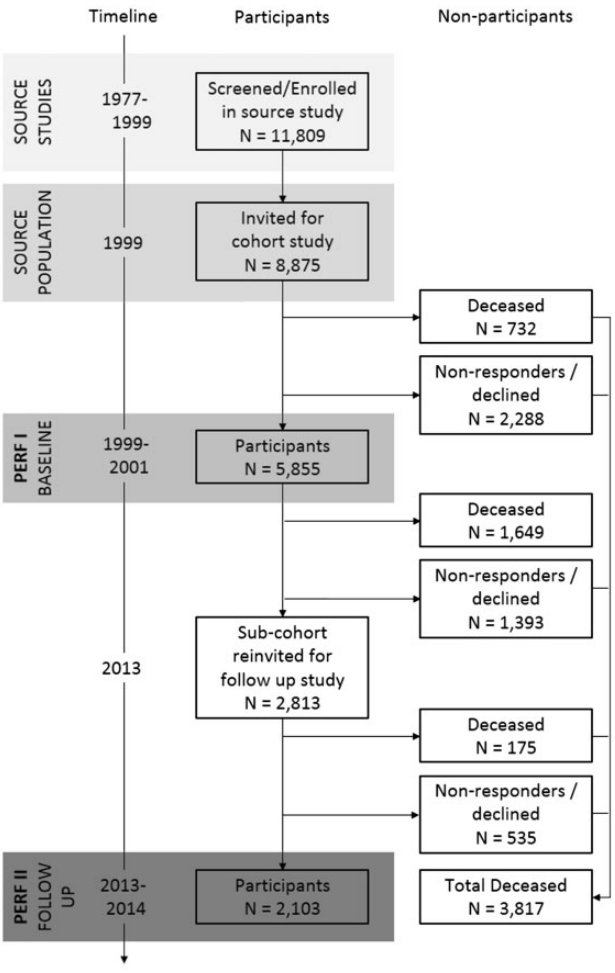

Figure 2. Flowchart of participants and >non-participants in the baseline and the follow-up study. The repeating occurrence of deceased and non-responders/declined illustrate the number of deceased and nonresponders/declined between two consecutive time points on the time scale.

Table 1. Selected baseline characteristics of the Prospective Epidemiological Risk Factor (PERF) study. The full study population ($n = 5855$) are shown along with specific subgroups of; subjects who died before follow-up ($n = 1649$), subjects who attended the follow-up visit (PERFII) (n = 2103) and subjects who did not attend the follow-up visit ($n = 2103$). Numbers are shown as absolute numbers with percentile in brackets for categorical variables. For numerical variables, the mean ± standard deviation (SD) are shown

Parameter	*N*	Baseline Participants (PERF I) *N* = 5855	Dead before follow-up *N* = 1649	Follow-up participants (PERF II) *N* = 2103	Follow-up non-participants *N* = 2103	***P*-value* Follow-up participants vs non- participants**
Age (mean ± SD, years)	5855	70.8 (6.5)	74.9 (5.9)	68.0 (6.0)	70.3 (5.9)	<0.001
Menopause age (mean ± SD, years)	5783	49.0 (4.9)	48.7 (5.0)	49.1 (4.8)	49.1 (4.8)	0.9
Highest level of education	5841					<0.0001
Primary school, *n* (%)		4178 (72)	1215 (74)	1428 (68)	1535 (73)	
High School, *n* (%)		1250 (21)	320 (20)	482 (23)	448 (21)	
University, *n* (%)		413 (7)	110 (7)	192 (9)	111 (5)	
Height (mean ± SD, cm)	5637	161 (5.9)	160 (6.0)	162 (5.8)	161 (5.8)	<0.001
Weight (mean ± SD, kg)	5637	67.7 (11.7)	65.5 (11.9)	68.8 (11.4)	68.4 (11.6)	0.2
BMI (mean ± SD, kg/m^2)	5637	26.1 (4.3)	25.7 (4.4)	26.2 (4.2)	26.5 (4.2)	0.07
BMI groups	5637					0.1
Underweight (<18.5), *n* (%)		90 (2)	46 (3)	21 (1)	23 (1)	
Normal (≥ 18.5-25.0), *n* (%)		2343 (42)	699 (45)	871 (42)	773 (38)	
Overweight (> 25.0-30.0), *n* (%)		2248 (40)	567 (37)	823 (40)	858 (43)	
Obese (> 30.0), *n* (%)		956 (17)	238 (15)	356 (17)	362 (18)	
Smoking	5844					0.6
Never, *n* (%)		2767 (47)	634 (39)	1077 (51)	1056 (50)	
Past, *n* (%)		1762 (30)	525 (32)	610 (29)	627 (30)	
Current, *n* (%)		1315 (23)	487 (30)	416 (20)	412 (20)	
Alcohol	5807					<0.0001
Never, n (%)		2531 (44)	757 (46)	843 (40)	931 (45)	
<10.5 alcohol units/week, n (%)		1380 (24)	348 (21)	451 (22)	581 (28)	
10.5-21 alcohol units/week, n (%)		1497 (26)	423 (26)	615 (29)	459 (22)	
>21 alcohol units/week, n (%)		399 (7)	107 (7)	180 (9)	112 (5)	
Physical activity	5843					0.05
Never, *n* (%)		1840 (31)	720 (44)	525 (25)	595 (28)	
1 time/week, *n* (%)		1233 (21)	340 (21)	451 (21)	442 (21)	
2 times/week, *n* (%)		748 (13)	179(11)	308 (15)	261 (13)	
3+ times/week, *n* (%)		2022 (35)	408 (25)	819 (39)	795 (38)	
Blood pressure						
Systolic (mean ± SD, mmHg)	5677	150 (24.4)	155 (25.4)	147 (23.3)	150 (24.2)	<0.001
Diastolic (mean ± SD, mmHg)	5679	81.9 (11.5)	81.7 (12.3)	82.0 (10.7)	81.8 (11.6)	0.6
Hypertension, *n* (%)	5838	1807 (31)	606 (37)	523 (25)	678 (32)	<0.0001
Hyperlipidaemia, *n* (%)	5845	530 (9)	142 (9)	224 (11)	164 (8)	0.002
Diabetes, *n* (%)	5842	181 (3)	75 (5)	47 (2)	59 (3)	0.06

*t test for numerical variables and chi-square test for categorical variables.

women attended the follow-up study (PERF II), which took place either in their own home or at the CCBR clinic in Ballerup. Figure 2 shows the number of participants and non-participants from baseline to follow-up. All the subjects were given ample time to consider participation and gave their written consent before any study-related procedure was carried out. The study was conducted in accordance with Good Clinical Practice and the Helsinki Declaration II.

Cohort characteristics, a comparison between baseline participants, follow-up participants and non-participants

The baseline characteristics of the entire cohort (PERF I) and the follow-up participants (PERF II) are shown in Table 1. The mean age in the baseline cohort (PERF I) was 70.8 years (49.7-88.8). Nearly 75% of the cohort had primary school as their highest level of education and less than 10% had a university degree. The follow-up

participants were characterized as being younger and slightly higher-educated. With an average BMI of 26.2 kg/m^2, this part of the cohort comprised 57% overweight or obese women. There were no differences between the follow-up participants and non-participants with regards to BMI. In relation to lifestyle variables (smoking, alcohol and physical activity), follow-up participants and non-participants for PERF II were found to be similar, although the follow-up participants comprised a higher proportion of subjects consuming > 10.5 alcohol units per week. The systolic blood pressure and the proportion of subjects with self-reported hypertension were higher in the group of non-participants than in the participating group, whereas the proportion of subjects with self-reported hyperlipidaemia was lower.

Cohort and target population characteristics

Comparison of study participants with the target population was done using data on Danish women aged 45+, from the Danish Health Interview Surveys (SUSY) in 2000[25] and 2005[26] and the StatBank from Statistics Denmark[27] (Table 2).

The average lifespan in the cohort is very similar to the average life span for Danish women. When compared with Danish women aged 45+ generally, the PERF cohort is characterized as slightly less physically active and more overweight/obese. The proportion of current smokers is less in the cohort and subjects not drinking alcohol is larger in our cohort compared with Danish women aged 45+. In relation to health, the two main causes of death are cardiovascular disease and cancer in both the cohort and the background population, and the proportions of deaths attributable to these diseases are comparable. For other comorbidities, the proportions of subjects with diabetes and depression in the cohort are similar to the target population, but the prevalences of hypertension and osteoporosis are approximately 2-fold higher in the cohort.

Table 2. Comparison of the PERF cohort and the target population comprising Danish women aged 45 and older. Data on the target population are derived from either Statistics Denmark or the Danish Health Interview Surveys. Values are shown as percentages if not otherwise indicated

Variable	Baseline cohort (PERF I)	Danish Women 45 + (target population)	*P*-value[e]
Demography and lifestyle			
Age (% of total group)			
60-64	18.3	25.3[a]	<0.01
65-69	23.2	22.0[a]	0.02
70-74	28.7	20.4[a]	<0.01
75-79	20.6	18.9[a]	<0.01
80-84	9.2	13.4[a]	<0.01
Average lifespan (years)[b]	83.0	82.7[a]	
Smoking (% of total group)			
Current	22.5	31.9[‡]	<0.01
Never	47.3	39.8[‡]	<0.01
Alcohol (% of total group)			
Never	43.6	28.2[c]	<0.01
<10.5 alcohol units/week	23.8	44.1[c]	<0.01
10.5-21 alcohol units/week	25.8	18.2[c]	<0.01
> 21 alcohol units/week	6.9	9.5[c]	<0.01
Physical activity (% of total group)			
No	31.5	21.9[c]	<0.01
Yes	68.5	78.1[‡]	<0.01
BMI (% of total group)			
Underweight (<18.5)	1.6	4.1[c]	<0.01
Normal weight (≥ 18.5 <25)	41.6	54.4[c]	<0.01
Overweight (≥ 25)	39.8	30.8[c]	<0.01
Obese (≥ 30)	17.0	10.7[c]	<0.01
Health			
Causes of death (% of total group)			
Cardiovascular	27.3	25.7[a]	
Cancer	32.2	33.8[a]	
Comorbidities (% of total group)			
Hypertension	31.0	16.4[c]	<0.01
Diabetes	3.1	3.9[c]	0.02
Osteoporosis	10.9	6.1[d]	<0.01
Depression/anxiety	6.6	5.5[d]	0.02

[a]Retrieved from Statistics Denmark.
[b]The average lifespan was calculated for all deceased subjects by the end of 2014.
[c]Data from the Danish Health Interview Surveys 2000.
[d]Data from the Danish Health Interview Surveys 2005.
[e]The z-score test for two population proportions.

How often have they been followed up?

Concomitant with the PERF II follow-up study, all subjects have been followed with registry linkage using population-based national registries. With approval from the authorities, we have collected registry data on all baseline participants ($n = 5855$). By use of a personal subject identification number (CPR-number), the Danish national registries contain individual-level data on the entire Danish population. Linkage has been done with the following registries: the National Danish Patient Registry, the National Danish Causes of Death Registry, the Danish National Diabetes Register, the Danish Cancer Registry and the Danish National Pathology Registry. For more information on the registries, please refer to Table 3.

The most recent linkage was done in January 2015, and this linkage is expected to continue until the remaining subjects from the cohort are deceased. The registry information is available for research within the scope of the study.

Table 3. Overview of registry linkage in the Prospective Epidemiological Risk Factor (PERF) study

Registry	Type of information received	Time period covered	Latest linking
National Danish Patient Registry	Hospitalization and discharge time	1977 on	31 Dec 2014
	Hospital and department	1977 on	
	Diagnoses (ICD classification)	1977 on	
	Treatments and operations	1996 on	
National Danish Causes of Death Registry	Time of death	1970 on	31 Jan 2015
	Underlying cause of death	1970 on	
	Complementary cause of death	1970 on	
Danish National Diabetes Register	Date of inclusion	1990 on	31 Dec 2014
	Inclusion criteria	1990 on	
	Inclusion cause	1990 on	
Danish Cancer Registry	Diagnosis and time of diagnosis	1943 on	31 Dec 014
	Tumour distribution	1943-2003	
	Treatment	1943-2003	
	TNM classification	2004 on	
	Ann Arbor staging	2004 on	
	Treatment	1943-2003	
Danish National Pathology Registry	Data from pathological tests (by SNOMED code)	1997 on (1970 on)	31 Dec 2014

What has been measured?

Baseline and follow-up examination

At the baseline visit (PERF I), participants completed a health examination involving a physical examination including blood pressure measurement, electrocardiogram (ECG), medical history and a health-related questionnaire (for more information on the questionnaire see separate section below). Participants provided blood and urine samples for standard biochemical analysis and for future analysis by storage in a biobank. Moreover, dual energy X-ray absorptiometry (DEXA) scans of the whole-body, spine, hip and arm, X-ray of the spine and mammography were obtained.

At the follow-up examination (PERF II), medical history and recording of all current medications were obtained. Measurements of height, weight, waist and hip circumferences, blood pressure, heart rate and respiratory frequency was completed. Muscle strength was determined using a hydraulic hand-grip dynamometer. An EQ-5D-3L evaluation was completed by the participant to assess their self-reported quality of life, and a Category Fluency Test together with a Short Blessed Test was done to test cognitive performance. Please refer to Table 4 for information on the data collected at the baseline and the follow-up examination.

Questionnaire

The baseline and follow-up questionnaire was completed as a structured interview with an investigator or study nurse and the participant. Standard demographic information such as age, menopause age and level of education, along with information on physical activity, current and past smoking habits and current and past drinking behaviour was included in the questionnaire. Information on diet obtained at baseline was limited to information on consumption of coffee/tea, dairy products and vegetarian status. Medical history, including treatment (medication/surgical) and familial medical history, was obtained as part of the interview for several disorders including, but not limited to, neurological or psychological disorders, cardio-/cerebrovascular disease, lung disorders, cancers, muscles and joint diseases and metabolic disorders.

Collection, analysis and storage of biological material

For each participant, urine and fasting blood samples were collected for routine analysis and biobank storage at baseline ($n = 5668$). The biobank also contains DNA samples for those subjects who gave written consent for this specific analysis ($n = 5553$). At the follow-up visit, fasting blood samples were collected. Samples are stored at -20 °C (urine, DNA samples) and -80 °C (serum). Routine blood and urine analysis was carried out at a College of American Pathology (CAP) certified central laboratory (Nordic Bioscience Laboratory) at both baseline and follow-up.

Genomics

In collaboration with deCODE genetics, Iceland, and Sct. Hans Hospital, Denmark, DNA samples from the PERF study have been genotyped and associations between single nucleotide polymorphisms (SNPs) and selected outcomes, including bone mineral density/osteoporotic fractures,[34]

Table 4. Parameters measured at the baseline (PERF I) and the follow-up visit (PERF II)

Parameter	Description	PERF I	PERF II
General information			
Demographics	Age	√	NA
	Body weight	√	√
	Height	√	√
	Education level	√	NA
Health			
Medical history	Self-reported questionnaire/interview	√	√
Physical examination	Full-body examination	√	—
	Blood pressure	√	√
	ECG	√	—
Cognition	Short Blessed Test	√	√
	Category Fluency Test (Animals)	√	√
Body composition	Arm, hip and spine DEXA	√	—
	Whole-body DEXA	√	—
X-ray	Spine	√	—
	Mammography	√	—
Muscle strength	Hand-grip strength test	—	√
Lifestyle			
Physical activity	Walking, leisure activity	√	√
Smoking	Current and past smoking behaviour	√	√
Alcohol	Current and past drinking behaviour	√	√
Diet	Consumption of coffee/tea, dairy products	√	—
	Vegetarians	√	—
Psychosocial parameters			
Quality of life, well-being	EQ-5D-3L[a]	—	√
Blood			
Haematology	Haemoglobin, leukocytes and differentiation, etc.	√	√
Lipids	Total cholesterol, LDL, HDL, triglycerides	√	√
Electrolytes	Sodium, potassium, calcium	√	√
Renal function	Creatinine	√	√
Liver	ALAT, ASAT, albumin, GGT, alkaline phosphatase	√	√
Inflammation	High sensitive CRP	—	√
Specialty biomarkers	Osteocalcin, CTX-1, VICM, C1M, C4M, TAU-C	√	*

NA, not applicable.

[a]EQ-5D-3L measures health in five dimensions (mobility, self-care, usual activities, pain/discomfort and anxiety/depression) and three levels (no problems, some problems, extreme problems).

type 2 diabetes,[35] schizophrenia,[36] depression[37] and cognitive impairment[38] have been assessed.

What has it found? Key findings and publications

The PERF study has generated several important findings covering the health of elderly women. Selected key findings are summarized in Table 5. In a cross-sectional nested analysis from PERF ($n = 1356$), it was shown that peripheral adiposity exhibits an independent anti-atherogenic effect in elderly women.[39,40] In the entire cohort and in a nested study ($n = 343$), it was shown that endogenous estrogen and hormone replacement therapy administered in the early phase of the menopause may have a protective association with cognitive impairment later in life.[41,42] More recently, it was shown that matrix metalloproteinase (MMP)-mediated collagen type I degradation, termed C1M, is an independent risk factor for all-cause mortality, as subjects with high levels of type I collagen degradation had a 2-fold increased mortality risk compared with subjects with low levels.[43] Last, a genome-wide association study of bone mineral density (BMD) among more than 30 000 subjects, including samples from PERF I, revealed a new BMD locus that harbours the PTCH1 gene. The gene is associated with reduced spine BMD.[44]

What are the main strengths and weaknesses?

In this 37-year ambidirectional population-based study, the participation rate has been higher than 70% throughout the study. To investigate whether the study population

Table 5. Summary of major findings from the Prospective Epidemiological Risk Factor (PERF) study

Endpoint/Exposure	Major findings
Cardiovascular disease	Localization of fat mass is more important for atherogenesis than obesity per se[39,40]
	Enlarged waist circumference and elevated triglycerides are simple diagnostic tools that could facilitate the identification of postmenopausal women at increased risk for accelerated atherogenesis and related adverse outcomes[45]
	Hormone replacement therapy for 2-3 years has relative cardiovascular benefits and reduces the risk of all-cause mortality[46]
Bone/osteoporosis	Limited hormone replacement therapy given in the early postmenopausal years can provide long-lasting benefits in terms of preventing bone loss and related fractures[47]
	Bone mass measurement offers effective fracture prediction independent of the site of measurement and age of the patient[48]
Association of conditions	Aortic calcification seems to independently contribute to the development of osteoporosis in the proximal femur[49]
	Independent association of peripheral vascular disease with osteoporosis in the proximal femur[50]
Cognitive function	Protective association of body fat mass with cognitive impairment in elderly women, through a more prominent exposure to endogenous estrogens[41]
	Short-term hormone replacement therapy administered in the early phase of the menopause may provide a long-term protection against cognitive impairment[42]
Genomics	Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes[35]
	Association of dopamine beta-hydroxylase gene variants with cognitive performance and depression in elderly women[37,38]
	Bone mineral density locus identified that harbours the PTCH1 gene. The gene is associated with reduced spine BMD[44]
All-cause mortality	Increased MMP-mediated tissue degradation, measured by C1M, is an independent risk factor for all-cause mortality[43]

resembled the target population, we compared the baseline participants with the general female population in Denmark aged 45 or older. It is well known that study participation is often linked to health superior to that of an otherwise similar, non-participant background population (healthy participant bias). However, we did not observe a healthier profile among the baseline study participants. The cohort is therefore considered representative of women aged 45+ in the Danish population. In the study population, we found a higher prevalence of osteoporosis at baseline. This could either be caused by selection bias, as a number of the participants had previously participated in randomized clinical trials focused on osteoporosis. However it could also reflect underdiagnosis of osteoporosis in the general Danish population, since the source population not only included women randomized for clinical studies but also those who did not meet the inclusion criteria (e.g. had high bone mineral density) at the time of recruitment.

Although the follow-up cohort (PERF II) was selected based on geographical limitations due to data collection in the participants' own homes, the similarities between follow-up participants and follow-up non-participants strengthen the internal validity of the data.

Besides the length of the follow-up period, the linkage to a range of nationwide registries is a major strength. The registry data are comprehensive and the registries were established relatively early, e.g. cancer and cause-specific death information since the 1940s and hospitalizations since the 1970s.[51] Registration has been mandatory since 1977. The registry data therefore strongly support the identification of outcomes and, because of the limited loss to follow-up, it adds analytical power to the study. Moreover, this cohort is to our knowledge one of the largest cohorts of postmenopausal women with full-body DEXA scans, which enables extensive studies of body composition.

Regarding weaknesses, the cohort only comprised women and therefore generalization cannot be made to men of similar ages. Moreover, the duration of time passed from PERF I (year 1999) to PERF II (year 2014) is long in a cohort of such advanced age. In order to prevent selection bias towards the healthier segment of this ageing cohort, great effort was made in following up on invited participants not instantly replying to our invitation. Also, visiting the subjects in their own homes increased the number of subjects with illnesses still wanting to participate.

Can I get hold of the data? Where can I find out more?

All data are stored electronically in anonymous form. Aliquoted biological material is stored in a biobank at the Nordic Bioscience Laboratory. Currently, the data are available only to employees of Nordic Bioscience A/S, Denmark; however, the PERF study group will welcome any enquiries regarding collaboration or data sharing for further investigations. Potential collaborators are invited to contact the PERF study group at [perf@nordicbio.com].

Profile in a nutshell

- The Prospective Epidemiological Risk Factor (PERF) Study is an ambidirectional population-based study of postmenopausal women set up with the purpose of obtaining a better understanding of the aetiology and pathogenesis of age-related diseases.
- Participants were recruited from a source population of 8875 women residing in Denmark. The baseline examination (PERF I) comprised 5855 women with mean age of 70.8 years (49.7-88.8) and took place between 1999 and 2001.
- All subjects have been followed up with registry linkage using population-based national registries. Further, a subcohort was re-invited to attend a follow-up visit between 2013 and 2014 (PERF II). Registry data are available for all baseline participants. From the baseline population, 2103 were enrolled in PERF II.
- The data repository contains a wide range of health-related and lifestyle measures, biological samples from the baseline and follow-up studies, genetic information and linkage to nationwide registries.
- The PERF study group will welcome any enquiries regarding collaboration or data sharing for further investigations.

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Conflict of interest: C.C. and B.J.R. serve as board members and stock owners in Nordic Bioscience A/S. M.A.K., J.R.A., P.Q. and A.B. hold stocks in Nordic Bioscience A/S.

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3.2 Cognitive Screening in PERF

The neuropsychological assessment in PERFI and PERFII included two short cognitive screening tools; the Short Blessed Test [171] and a category fluency test with animal naming [172]. The characteristics of each test, including operationalization and diagnostic performance in relation to dementia is outlined below.

3.2.1 The Short Blessed Test

The Short Blessed Test (SBT) is a six-item test assessing orientation, concentration, and memory. Scores range from 0 to 28, with lower scores indicating better performance. The SBT includes three questions related to orientation (Q1: What year is it now? Q2: What month is it now? Q3: What time is it now?), followed by two questions related to concentration (Q4: counting backwards from 20 to 1 and Q5: listing the 12 months backwards within 1 minute). Finally, in relation to memory, subjects are asked to repeat and remember a memory phrase (a name and an address) following the two first questions (Q1 and Q2). The memory phrase should be recalled after Q5. The administration time is approximately 5 minutes. Findings from the Memory and Aging Project suggest that a score between 0-4 is considered normal while 5-9 is consistent with questionable impairment (sometimes also referred to as MCI). A threshold of ≥10 was identified as cognitive impairment consistent with dementia [173]. The diagnostic accuracy is very comparable, occasionally superior, to more comprehensive tests like the Mine Mental State Examination when it comes to dementia screening [174–176].

3.2.2 Category Fluency Test

The category fluency test (CFT) measures verbal fluency, in this test subjects are asked to name as many animals as possible in 60 seconds. Higher scores indicate better performance [172]. The administration time is 1-2 minutes. Different fluency tests are widely used in neuropsychological testing for dementia, with the category of “animals” as the most frequently employed [177]. In relation to the separation of AD subjects from cognitive normal individuals, receiver operating characteristic curve analysis has found the diagnostic accuracy to be excellent (AUC > 0.9) [178,179]. An imaging study has shown that the temporal lobe is activated while performing the test [180]. Few studies have examined the prognostic performance of the test although category fluency has been shown to be able to discriminate between very mild AD and controls [181].

3.3 Dementia in PERF

3.3.1 The Danish health registries

Dementia diagnosis was obtained from two Danish national health registries: The Danish National Patient Register (NPR) and the Danish Register of Causes of Death (RCD). The registries contain individual level data on the entire Danish population. Cause of death registration dates back to

1875 and since 1970 it has been fully computerised. In its current form, a cause of death is registered by the medical doctor who issues a death certificate indicating the underlying and contributory causes of death. The causes have been classified according to ICD-10 since 1994 [182]. The NPR was established in 1977 and is considered the most comprehensive of its kind. All diagnoses have been classified in accordance with ICD-10 since 1994. Before 1994 diagnostic information was coded according to ICD-8 [183]. The following codes were considered a dementia diagnosis: F00-F03, G30-G32 and R54.

3.3.2 Diagnostic groups in PERF

In total 636 incident dementia cases were identified from the NPR and RCD during the follow-up period. As outlined in table 5 all dementia cases were divided into three differential groups. The majority of subjects holding a dementia diagnosis was identified from the NPR (n = 581) while the remaining 55 dementia cases were identified solely on their cause of death in RCD, since they were not diagnosed with dementia according to the NPR.

Table 5: Overview of dementia diagnosis in PERF grouped by ICD-10 and study specific diagnostic groups.

Study specific group	ICD-10 code	Description	N
AD	F00	Dementia in Alzheimer disease	264
	G30	Alzheimer disease	
VaD	F01	Vascular dementia	47
OD	F02	Dementia in other diseases classified elsewhere	325
	F03	Unspecified dementia	
	G31	Other degenerative diseases of nervous system, not elsewhere classified	
	G32	Other degenerative disorders of nervous system in diseases classified elsewhere	
	R54	Senility	
All-cause dementia	All	All above	636

3.3.3 Incidence of dementia

The overall dementia incidence in the cohort was 8.3 per 1000 person years from baseline until the end of the follow-up period. The follow-up started on the day of study enrolment and ended at occurrence of event (dementia diagnosis), death, or on December 31, 2014 (retrieval of registry data), whichever came first. The age specific incidence rates increased with increasing age ranging from 1.1 per 1000 person years in the youngest age group (<60 years) to 26.8 per 1000 person years in the oldest age group (≥80 years). The incidence approximately doubles every 5 year from age 60 to 80 (Figure 9).

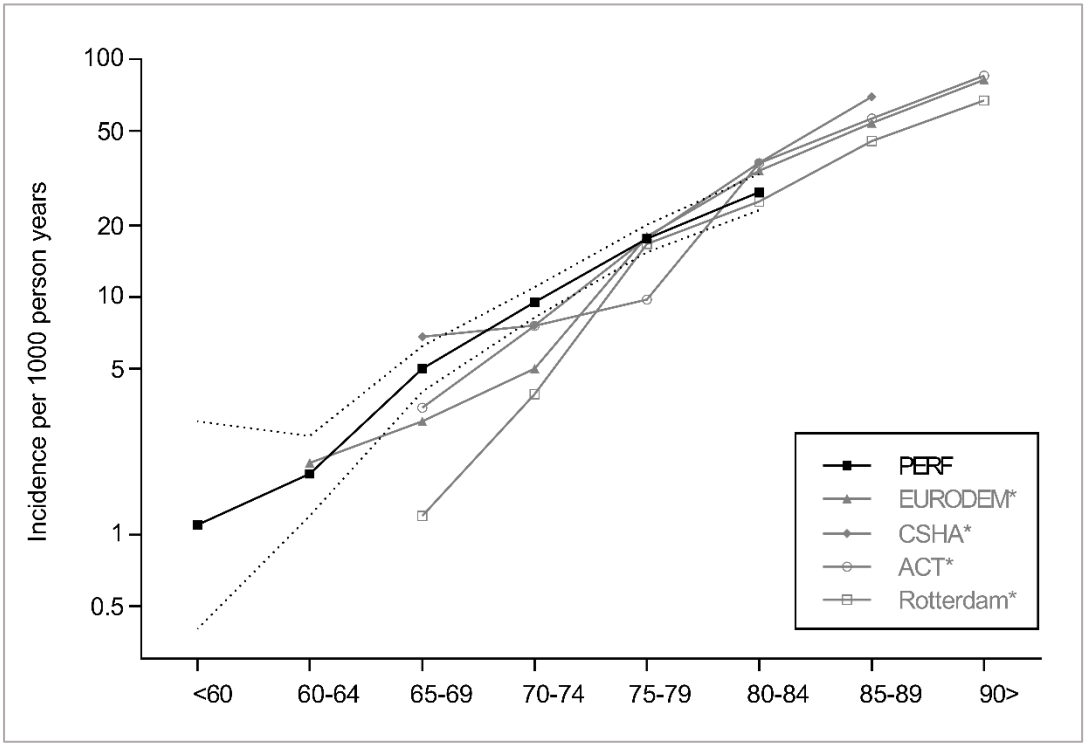

Figure 9: Age stratified incidence rates in the PERF cohort compared to other cohorts of relevance [35,184–186]. Dotted lines represent the 95% confidence limits. *Only incidence for women is illustrated. EURODEM: Pooled analysis of four population-based prospective cohort studies. CSHA: The Canadian Study of Health and Aging. ACT: Adult Changes in Thought study. Rotterdam: The Rotterdam Study.

This section concludes the introductory and methodological part of the thesis. The following section contain each of the four remaining original research papers followed by a general discussion and finally a few concluding remarks.

4

Late-Life Risk Factors for All-Cause Dementia and Differential Dementia Diagnoses in Women

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Late-Life Risk Factors for All-Cause Dementia and Differential Dementia Diagnoses in Women

A Prospective Cohort Study

Jesper Skov Neergaard, MSc, Katrine Dragsbæk, MSc, Henrik Bo Hansen, MSc, Kim Henriksen, PhD, Claus Christiansen, MD, PhD, and Morten Asser Karsdal, PhD

Abstract: Since the first evidence of a decline in dementia incidence was reported in 2011, the focus on modifiable risk factors has increased. The possibility of risk factor intervention as a prevention strategy has been widely discussed; however, further evidence in relation to risk factors is still needed.

The Prospective Epidemiologic Risk Factor (PERF I) study was an observational prospective study of postmenopausal Danish women who were initially examined between 1999 and 2001 (n = 5855). Follow-up data on diagnosis and survival as of December 31, 2014 was retrieved from the National Danish Patient Registry and the National Danish Causes of Death Registry. Cox proportional hazards regression model was applied to calculate adjusted hazard ratios (HR) for selected risk factors for dementia.

Of 5512 eligible subjects, 592 developed dementia within the follow-up period of maximum 15 years. The independent factors associated with increased risk of all-cause dementia were depression (HR = 1.75 [95% CI 1.32–2.34]) and impaired fasting glucose levels. A dose–response relationship was observed between fasting glucose level and risk of dementia with HRs of 1.25 [1.05–1.49] and 1.45 [1.03–2.06] for impaired (5.6–6.9 mmol/L) and hyperglycemic (≥7.0 mmol/L) glucose levels, respectively. The factors associated with a decreased risk of dementia were overweight in late-life (HR = 0.75 [0. 62–0.89]) and physical activity at least once weekly (HR = 0.77 [0.61–0.96]).

The identified risk factors for dementia in women in late-life are all considered modifiable. This supports the notion that prevention strategies may improve the poor future prospects for dementias in the ageing population.

(*Medicine* 95(11):e3112)

Abbreviations: AD = Alzheimer disease, APOE = apolipoprotein E, BMI = body mass index, CI = confidence interval, HR = hazard ratio, OD = other/unspecified dementia, PERF = the Prospective Epidemiologic Risk Factor study, VaD = vascular dementia.

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From the Nordic Bioscience A/S, Herlev, Denmark.
Correspondence: Jesper Skov Neergaard, Nordic Bioscience A/S, DK-2730 Herlev, Denmark (e-mail: jsn@nordicbioscience.com).
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INTRODUCTION

The world's population is ageing. As a result, the prevalence and incidence of dementia has escalated. From the most recent projections, the prevalence and thereby total number of people with dementia is projected to nearly triple by 2050 reaching a total of 131.8 million people worldwide, driven almost entirely by prolonged longevity.[1] Since the first signs of a potential decline in dementia incidence in the United States were published in 2011,[2] followed by several other studies from Europe,[3–5] the possibility of primary prevention by addressing risk factors has been widely discussed.

Risk factors for dementia are divided into the nonmodifiable and modifiable. The nonmodifiable or genetic risk factors include the Apolipoprotein E (APOE) ε4 allele, age, and female sex.[6–8] Many modifiable risk factors have been suggested, but despite extensive research efforts the evidence is inconclusive. In 2010, the National Institutes of Health in the United States stated that results from previous studies were not of sufficient strength to warrant specific recommendations for disease prevention.[9] In 2014, the Alzheimer's Association reached a similar conclusion stating that there is still significant uncertainty with respect to the relationship between individual risk factors and dementia,[10] justifying the need for further studies.

It is estimated that around one-third of Alzheimer disease cases worldwide are caused by 7 modifiable risk factors; low educational attainment, physical inactivity, smoking, midlife hypertension, midlife obesity, diabetes, and depression.[11] Further evidence from the FINGER study, a randomized clinical trial in Finland, suggests that a multidomain interventional approach focusing on several modifiable risk factors can improve or maintain cognitive function in the elderly population.[12]

Our objective was to investigate late-life risk factors for dementia among elderly women. The women comprised the PERF cohort in Denmark, one of the largest individual prospective cohorts of elderly women. The outcome, dementia, was assessed a maximum of 15 years after baseline.

METHODS

Study Population

The Prospective Epidemiologic Risk Factor (PERF I) study was an observational, prospective follow-up study of Danish postmenopausal women. The study participants were identified from a database of subjects who had previously been screened for participation in 1 of 21 clinical randomized controlled trials initiated between 1977 and 1996, including both intervention and nonintervention studies. A total of 8875 women constituted the source population, of which 5855 women gave their written informed consent to participate in the PERF I cohort study. There were no in/exclusion criteria at

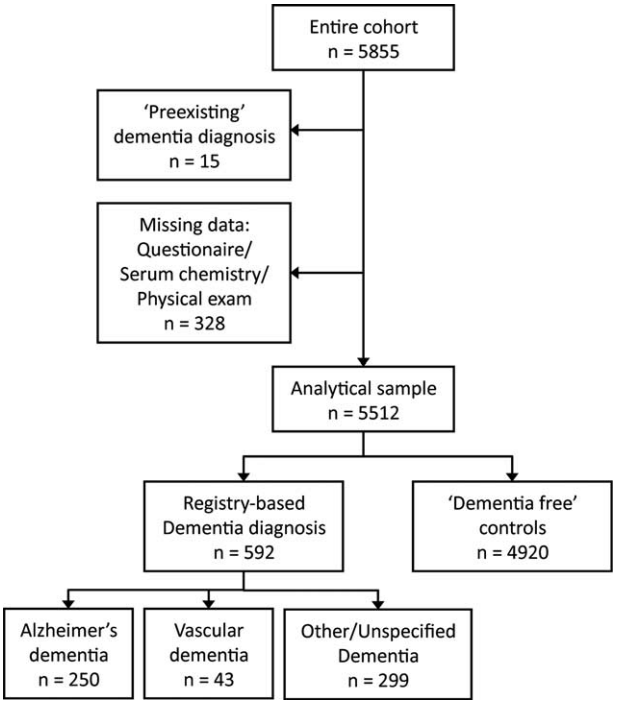

FIGURE 1. Analytical sample for the assessment of risk factors for all-cause dementia and differential dementias: Alzheimer dementia, vascular dementia, and other/unspecified dementias.

the time of enrolment in the observational study. The baseline examination took place between 1999 and 2001 and comprised an interview with completion of a predefined questionnaire, a physical examination, and blood sampling at the study site. The questionnaire was completed by 5847 subjects. Subject's medical history including, but not limited to, history of depression, history of cerebral embolism/hemorrhage, history of hypertension and current treatment, history of diabetes and current treatment and hyperlipidemia and current treatment, were self-reported as part of the questionnaire. The physical examination was completed by 5677 subjects. Vital signs including height, weight measured without shoes but with indoor clothes and blood pressure were measured on calibrated equipment. Blood samples were taken from 5668 subjects and analyzed at a central laboratory. The analytical sample was defined as subjects with no missing data on all relevant variables as illustrated in the flow diagram (Figure 1). The study was carried out in accordance with ICH-GCP with study protocol approval from local ethics committees.

Dementia Endpoint

Follow-up information on dementia status and survival as of December 31, 2014 was retrieved from the National Danish Patient Registry and the National Danish Causes of Death Registry using a unique personal identification number for each subject. The follow-up started on the day of study enrollment and ended at occurrence of event (dementia diagnosis), death, or on December 31, 2014 (retrieval of registry data), whichever came first. Of the entire study population, a total of 651 dementia cases were identified from the registries. Fifteen subjects were excluded from the analysis due to a dementia diagnosis prior to study enrollment. Fifty-five cases were identified based solely on their cause of death in the National Danish Causes of Death Registry, since they were not diagnosed with dementia according to the National Danish Patient Registry. The remaining subjects (n = 581) had a diagnosis of dementia in the National Danish Patient Registry leading to a total of 636 incident dementia cases prior to identification of the analytical sample. Dementia diagnoses were classified according to the International Classification of Diseases, 10th revision (ICD10). The following codes were considered a dementia diagnosis: "OD" (dementia in other diseases classified elsewhere; unspecified dementia and senility) [F02-F03 and R54, n = 325], "AD" (dementia in Alzheimer disease, other degenerative diseases of the nervous system) [F00 and G30–G32, n = 264], and "VaD" (vascular dementia) [F01, n = 47].

Statistical Analysis

Statistical analyses were conducted using MedCalc Statistical Software version 14.8.1 (MedCalc Software bvba, Ostend, Belgium) and SAS version 9.4 (SAS Institute Inc, Cary, NC).

Baseline characteristics of controls and subjects found to have dementia at follow-up were compared using a one-way analysis of variance (ANOVA) for quantitative variables and χ^2 test for comparison of categorical variables (Table 1).

A Cox proportional hazards regression model was used to assess the selected risk factors in an age-adjusted and a multivariate adjusted regression analysis, the follow-up time since baseline was used as time scale. Age was included as continues variable and risk estimates reported pr. 5 years of aging. In the multivariate model, the categorical variables education level (primary school, high school, or university), body mass index (BMI, kg/m^2) where underweight was <18.5, normal weight $\geq 18.5 < 25$, overweight $\geq 25 < 30$, and obese ≥ 30, smoking (never, past, or current), alcohol consumption (never, <10.5 alcohol units/week, 10.5–21 alcohol units/week, or >21 alcohol units/week), physical activity (other than walking) (never, once weekly, twice weekly, or 3 or more times per week), history of depression (yes/no), history of cerebral embolism/hemorrhage (yes/no), systolic blood pressure >160 mm Hg, fasting glucose levels (normal <5.6 mmol/L, impaired 5.6–6.9 mmol/L, or hyperglycemic ≥ 7.0 mmol/L) and total cholesterol levels >6.5 mmol/L and age as a continuous variable were included. Subjects who reported treatment for hypertension, diabetes, or hyperlipidemia at baseline were included in the hypertensive (systolic blood pressure >160 mm Hg), hyperglycemic (fasting glucose ≥ 7.0 mmol/L), or hyperlipidemic (total cholesterol levels >6.5 mmol/L) groups, respectively. Regression analysis was performed for all-cause dementia and separate analyses for differential diagnoses (OD, AD, and VaD). Due to a large proportion of missing data from 781 subjects, the family history of dementia (yes/no) was not included in the multivariate analysis.

RESULTS

Baseline Characteristics

Of the analytical sample (n = 5512), a total of 592 dementia cases were identified from the registries during the follow-up period (Table 1). The maximum follow-up period was 15 years (mean follow-up: 11.9 ± 3.9 years) starting on the day of study enrollment and ending at occurrence of event (dementia

TABLE 1. Demographic Characteristics by Dementia Status of the Analytical Sample at Baseline

Variable	Dementia-Free Controls n = 4920	All-Cause Dementia n = 592	Alzheimer's Dementia n = 250	Vascular Dementia n = 43	Other/ Unspecified Dementia n = 299	P-value All-cause dementia vs. control	P-value Differential diagnosis vs. control
Age, mean ± SD, yr	70.1 ± 6.4	75.1 ± 5.3	74.4 ± 4.9*,†	74.3 ± 5.8*	75.8 ± 5.5*,‡	<0.001	<0.001
All-cause deaths until December 31, 2014, n (%)	1388 (28)	386 (65)	141 (56)	27 (63)	218 (73)	<0.001	<0.001
Education, n (%)							
Primary school	3496 (71)	437 (74)	186 (74)	36 (84)	215 (72)		
High school	1072 (22)	111 (19)	44 (18)	6 (14)	61 (20)	0.2	0.4
University	352 (7)	44 (7)	20 (8)	1 (2)	23 (8)		
BMI, mean ± SD, kg/m^2	26.2 ± 4.2	25.8 ± 4.2	25.8 ± 4.2	26.8 ± 5.4	25.7 ± 4.1	0.06	0.1
Smoking, n (%)							
Never	2334 (47)	284 (48)	129 (52)	14 (33)	141 (47)		
Past	1477 (30)	196 (33)	72 (29)	16 (37)	108 (36)	0.09	0.04
Current	1109 (23)	112 (19)	49 (20)	13 (30)	50 (17)		
Alcohol consumption, n (%)							
Never	2115 (43)	282 (48)	122 (49)	14 (33)	146 (49)	0.2	0.3
<10.5 alcohol units/week	1174 (24)	134 (23)	58 (23)	13 (30)	63 (21)		
10.5–21 alcohol units/week	1283 (26)	142 (24)	58 (23)	12 (28)	72 (24)		
>21 alcohol units/week	348 (7)	34 (6)	12 (5)	4 (9)	18 (6)		
Physical activity							
None	1469 (30)	227 (38)	81 (32)	22 (51)	124 (42)	<0.001	<0.001
1 time/week	1049 (21)	118 (20)	48 (19)	8 (19)	62 (21)		
2 times/week	639 (13)	74 (13)	35 (14)	4 (9)	35 (12)		
3+ times/week	1763 (36)	173 (29)	86 (34)	9 (21)	78 (26)		
Systolic blood pressure >160 mm Hg or treated hypertension (self-reported)	1852 (38)	275 (47)	103 (41)	20 (47)	152 (51)	<0.001	<0.001
History of cerebral embolism/hemorrhage (self-reported)	142 (3)	28 (5)	10 (4)	1 (2)	17 (6)	0.02	0.04
Fasting glucose							
Normal (<5.6 mmol/L)	3181 (65)	349 (59)	146 (58)	25 (58)	178 (60)	0.02	0.3
Impaired (5.6–6.9 mmol/L)	1486 (30)	206 (35)	89 (36)	15 (35)	102 (34)		
Hyperglycemic (≥7.0 mmol/L) or treated diabetes (self-reported)	253 (5)	37 (6)	15 (6)	3 (7)	19 (6)		
Total cholesterol (>6.5 mmol/L) or treated hyperlipidemia (self-reported)	2138 (44)	287 (49)	125 (50)	20 (47)	142 (48)	0.02	0.2
History of depression (self-reported)	307 (6)	52 (9)	12 (5)	4 (9)	36 (12)	0.02	0.0006
History of other neural disorders (self-reported)§	1601 (33)	229 (39)	92 (37)	22 (51)	115 (50)	0.003	0.006

* Significant difference ($P < 0.05$) from dementia-free controls (pairwise comparison using Student–Newman–Keuls test).
† Significant difference ($P < 0.05$) from other/unspecified subgroup (pairwise comparison using Student–Newman–Keuls test).
‡ Significant difference ($P < 0.05$) from Alzheimer disease subgroup (pairwise comparison using Student–Newman–Keuls test).
§ Other neural disorders include chronic headache, eye disorders, disabled hearing, and epilepsy.

diagnosis), death, or on December 31, 2014 (retrieval of registry data), whichever came first.

The dementia groups (AD, VaD, and OD) were characterized as being markedly older than dementia-free controls (74.4–75.8 versus 70.1 years, $P < 0.001$). The proportion of deceased subjects in each dementia group was markedly higher than in the dementia-free control group. No differences were observed in education levels ($P = 0.2$), BMI ($P = 0.06$), smoking habits ($P = 0.09$), and alcohol consumption ($P = 0.2$) when comparing all-cause dementia with dementia-free controls. The dementia groups are characterized by a larger proportion of subjects with elevated blood pressure ($P < 0.001$) and a larger proportion of physically inactive subjects ($P < 0.001$). When comparing the differential groups with the dementia-free controls, smoking habits, physical activity, elevated blood pressure, history of cerebral embolism/hemorrhage, history of depression, and other neural disorders were significantly different. No significant differences were observed in the proportion of subjects with hyperlipidemia between the differential dementia groups and the dementia-free controls ($P = 0.2$).

Risk Factors for All-Cause Dementia

The overall incidence of dementia in the analytical sample was 8.9 (8.3–9.7) per 1000 person years. The age-specific incidence rates increased with increasing age ranging from 0.9 (0.3–2.7) per 1000 person years in the youngest age group (<60) to 28.0 (23.4–33.6) per 1000 person years in the oldest age group (≥80). The incidence approximately doubled every 5 year (data not shown).

A Cox proportional hazards regression model was used to assess HRs for selected risk factors as listed in Table 2.

Age was a strong risk factor for all-cause dementia and for differential diagnoses. From an age-adjusted model, physical activity (other than walking) at least once a week and overweight were associated with decreased risk of all-cause dementia, while depression and higher levels of fasting glucose (≥5.6 mmol/L) were associated with an increased risk of dementia (see Table 1, Supplemental Content, which contains the results from the age-adjusted model, http://links.lww.com/MD/A780).

In the multivariate analysis the independent factors associated with increased risk of dementia were depression, impaired fasting glucose levels (5.6–6.9 mmol/L), and hyperglycemia (>6.9 mmol/L or treated diabetes). The factors associated with a decreased risk were overweight and physical activity (other than walking) at least once a week. Obesity as defined by a BMI ≥30 was not associated with the development of dementia (Table 2).

No major differences were observed between the age-adjusted and the multivariate-adjusted models.

Risk Factors for Differential Dementia Diagnosis

The risk factor profiles for differential diagnoses of dementia were generally similar but certain risk factors were notably different between the AD, VaD, and OD groups (Table 2). The age-adjusted models revealed that family history of dementia was associated with an increased risk of AD but no association was observed for VaD and OD. Impaired fasting glucose levels were solely associated with AD in the multivariate adjusted model, increasing the risk by 33% compared with normal glucose levels. Being overweight had a negative association with both AD and OD, lowering the risk by 28% and 25% respectively in the multivariate analysis. Physical activity at least 3 times per week was associated with a decreased risk of VaD (58%) and OD (29%) compared with those being physically inactive (apart from walking). Smoking increased the risk of VaD, in which the risk was 156% higher than in subjects who had never smoked. Depression increased the risk for OD with a similar magnitude as smoking did for VaD. No association was observed between depression and AD or VaD in either of the regression models (Table 2).

DISCUSSION

Using public health registries we were able to follow subjects for up to 15 years from baseline, providing an excellent opportunity to study potential risk factors in a large sample of elderly women. To our knowledge, this is one of the largest individual prospective cohort studies to investigate risk of all-cause dementia and differential dementia diagnoses in late-life.

Equal to our findings, other large cohort studies (including the EURODEM collaboration) have found incidence rates of dementia for women comparable to what we found in the PERF cohort.[7,13,14]

The factors associated with an increased risk of all-cause dementia were increasing age, physical inactivity, depression, and impaired glucose levels. Being overweight in late-life was protective against development of all-cause dementia when compared with women with a normal BMI. The differential diagnoses of dementia shared several risk factors. Smoking and depression were solely associated with a higher risk of developing VaD and OD, respectively.

Our results suggest that overweight in women (mean age 70.7, SD 6.5) has a protective relation to development of all-cause dementia, AD, and OD. Overweight and obesity have previously been linked to dementia in both midlife and late-life. A BMI in midlife indicating overweight or obesity has often been proposed to increase risk of developing dementia in later life.[15,16] Evidence suggests that the association between overweight/obesity and dementia vanish later in life.[15] A study in late-life from the Kungsholmen cohort in Sweden (mean age 80.8, SD 4.5) showed, separately for both men and women, a similar negative relationship between high BMI and development of dementia as we found in our study.[17] The CAIDE study in Finland also showed a negative association between high BMI in late-life and development of dementia.[18] Contradictorily, a retrospective cohort study involving nearly 2 million men and women in the UK recently disproved the hypothesis that obesity in midlife could increase the risk of dementia in later life and actually strengthened the evidence that overweight and obesity may protect against dementia in later life.[19]

The CAIDE study also showed that a decrease in BMI from mid- to late-life and a low late-life BMI of <25 kg/m^2 (mean age, 71.2, SD 4.0) are associated with higher risk of all-cause dementia and AD.[18] We have also previously shown an association between changes in body fat mass and cognitive impairment in elderly women.[20] The relationship is however unlikely to be causal since weight loss is known to occur with comorbidities in late-life, and is therefore often linked to poor health and mortality.[21] In addition, BMI is known to have several limitations as a health measure,[22] wherefore a simple measure like waist circumference would have been of interest in the evaluation of bodyweight and body composition in relation to dementia.

Among the lifestyle factors studied, only physical inactivity had an association with increased risk of all-cause dementia. Physical activity at least once weekly reduced the risk of all-cause dementia by 20% to 23% compared with physical

TABLE 2. Multivariate-Adjusted Hazard Ratios (HRs) for Risk Factors Associated With All-Cause Dementia and Differential Dementia Diagnoses

	All-Cause Dementia		Alzheimer's Disease		Vascular Dementia		Other/ Unspecified Dementia	
Variable	**HR**	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**
Demographics								
Age (per 5 years of ageing)	2.05	1.89–2.21	1.92	1.71–2.15	1.85	1.41–2.43	2.32	2.07–2.60
Education								
Primary school	Reference							
High school	0.91	0.74–1.13	0.84	0.60–1.17	0.52	0.22–1.26	1.05	0.78–1.40
University	0.91	0.66–1.25	0.98	0.61–1.57	0.23	0.03–1.67	0.96	0.62–1.50
BMI								
<18.5 (underweight)	0.88	0.45–1.72	0.92	0.34–2.51	no data		0.93	0.38–2.28
≥18.5 <25 (normal)	Reference							
≥25 <30 (overweight)	0.75	0.62–0.89	0.72	0.54–0.96	0.68	0.33–1.40	0.75	0.58–0.98
≥30 (obese)	0.79	0.62–1.01	0.74	0.51–1.09	1.28	0.57–2.86	0.75	0.52–1.06
Lifestyle								
Smoking								
Never	Reference							
Past	1.14	0.95–1.37	0.93	0.70–1.25	1.71	0.83–3.54	1.28	0.99–1.65
Current	1.13	0.90–1.41	1.08	0.77–1.51	2.56	1.18–5.55	1.04	0.74–1.44
Alcohol								
Never	1.09	0.89–1.35	1.12	0.82–1.53	0.53	0.25–1.13	1.19	0.89–1.61
<10.5 alcohol units/week	Reference							
10.5–21 alcohol units/week	0.97	0.76–1.23	0.91	0.63–1.32	0.90	0.41–2.00	1.03	0.73–1.45
>21 alcohol units/week	0.97	0.67–1.42	0.81	0.43–1.52	1.13	0.36–3.50	1.07	0.63–1.82
Physical activity								
None	Reference							
1 time/week	0.77	0.61–0.96	0.84	0.58–1.20	0.55	0.24–1.25	0.76	0.56–1.04
2 times/week	0.80	0.61–1.04	0.99	0.66–1.47	0.46	0.16–1.35	0.72	0.49–1.05
3+ times/week	0.79	0.64–0.97	1.00	0.73–1.37	0.42	0.19–0.93	0.71	0.53–0.95
Vascular								
Systolic blood pressure >160 mm Hg or treated hypertension	1.05	0.89–1.24	0.89	0.69–1.16	1.16	0.62–2.16	1.18	0.93–1.49
History of cerebral embolism/hemorrhage	1.28	0.88–1.88	1.17	0.62–2.22	0.57	0.08–4.20	1.43	0.87–2.34
Fasting glucose/diabetes								
Normal (<5.6 mmol/L)	Reference							
Impaired (5.6–6.9 mmol/L)	1.25	1.05–1.49	1.33	1.02–1.74	1.23	0.63–2.36	1.20	0.94–1.54
Hyperglycemic (≥7.0 mmol/L) or treated diabetes	1.45	1.03–2.06	1.56	0.90–2.69	1.35	0.39–4.71	1.47	0.91–2.39
Total cholesterol (>6.5 mmol/L) or treated hyperlipidemia	1.12	0.95–1.32	1.19	0.93–1.53	1.09	0.60–2.00	1.07	0.85–1.34
Neural disorders								
History of depression (yes/no)	1.75	1.32–2.34	0.96	0.53–1.71	1.86	0.66–5.26	2.58	1.82–3.68
History of other neural disorders (yes/no)	0.99	0.83–1.17	0.97	0.75–1.27	1.67	0.91–3.08	0.93	0.73–1.18

All HRs listed in the table were mutually adjusted.

inactivity. For the differential diagnoses of dementia, physical inactivity was associated with risk of VaD and OD. The causal relation between physical activity and dementia is uncertain and some suspect the length of the follow up period may have biased some of the previous findings.[23] A study of physical activity in late-life from the Rotterdam cohort put follow-up time into perspective.[24] The investigators suggest that physical activity has an inverse relationship with dementia onset during up to 4 years of follow-up, after which the protective effect diminishes. They speculate this may either be related to reverse causation or a short-term effect of physical activity.[24] An increase in physical activity after midlife recently was shown to protect against both all-cause dementia and AD,[25] supporting the association observed in the current study.

Smoking was not related to all-cause dementia in our cohort. However, in the analysis of differential diagnoses, current smoking was associated with an increased risk of VaD. Pathologically, this makes sense since smoking is a strong risk factor for both cerebrovascular and cardiovascular diseases. Smoking is involved in atherosclerosis, causing narrowing of blood vessels in the brain. In addition, smoking has been shown to have both a direct, affecting the folding of amyloid β, and an indirect detrimental effect in relation to dementia.[26,27]

Depression increased the risk of all-cause dementia and OD. Evidence from the literature is consistent with our findings where late-life depression has been associated with the development of dementia.[28,29] The most recent meta-analyses, one in the 2014 World Alzheimer's Report[3] and another from Diniz et al,[30] reported increased risks of 97% and 85% respectively. In the present study, the risk of developing all-cause dementia increased by 75% in elderly women with a history of depression, compared with subjects who had never suffered from the illness. The causal relationship between depression and dementia is however unclear. In the current study, we have no information about the onset of depressive symptoms. In the case of late-life onset, the observed association could potentially be a result of reverse causation.

There is somewhat more limited evidence when it comes to depression and risk of differential dementia diagnoses. In the current study, we found an association with OD (HR 2.58 (95% CI 1.82–3.68), while no association was observed with AD and VaD. Barnes et al[28] studied all-cause dementia, AD, and VaD and found associations between both AD and VaD for subjects with either late-life depressive symptoms or subjects with both midlife and late-life symptoms. The review from Diniz et al[30] suggests similar associations in their pooled estimates with the strongest association between depression and VaD. The missing association with AD in the current study may be caused by misclassification of subjects in the OD group—a heterogeneous group that is likely to contain several subjects with AD and mixed pathologies.

Our findings suggest a potential dose–response relationship between fasting glucose levels and risk of all-cause dementia when measured in late-life. The risk of all-cause dementia was increased by 25% and 45% in the impaired and hyperglycemic groups, respectively. The association between self-reported diabetes and risk of dementia did not confirm this relation, a potential result of under diagnosis which has been estimated to be up to 46% worldwide.[31] In relation to diabetes increased risks of 50% and 58% have previously been reported in the Kungsholmen Study and the French Three-City Study.[32,33] Contrarily, the Three-City Study found no association between impaired fasting glucose and dementia only with diabetes.

STRENGTHS AND LIMITATIONS

The follow-up information derived from registry data is uniquely available in Denmark where all contacts with primary care have been registered since 1977. This results in very limited loss to follow-up and all subjects can be followed up until time of death. We studied a large group of elderly women in Denmark, a homogenous population where generalization to other population is not obvious. The cohort only comprised women and therefore generalization cannot be made to men of similar ages. It is well known that women are at higher risk for developing dementia and although some risk factors are likely to be determined by the population in study, the HRs from Cox proportional-hazard analysis were comparable to associations found in similar cohorts making the generalization more likely.

Among the limitations of the study is the missing Apolipoprotein E (APOE) assessment. The APOE ε4 allele is a major genetic risk factor for AD.[6] Further, we did not include any measures of cognitive performance or activities of daily living at baseline in this analysis, and since we did not have screening for dementia using a standard diagnostic criteria at baseline it is possible that some of the dementia cases had prodromal disease already at baseline eventually affecting the cause and effect relationship. Risk factors assessed in the analysis were selected based on the available data and evidence from the literature. No measures of nutrition or information on diet were obtained at baseline. These factors have previously been suggested as risk factors for dementia and could potentially introduce residual confounding in our analysis.[34,35]

Epidemiological study designs such as that of the PERF I study may introduce selection bias by possible over-representation of relatively healthy subjects in the cohort. Participants in the PERF I study were recruited by active recruitment from the CCBR Clinical Research subject database, a recruitment method that could lead to above-mentioned selection bias. It should however be noted that their where no in- or exclusion criteria's at the time of enrolment, which could potentially reduce the risk of selection bias.

In relation to differential diagnosis the method with registry-linkage may have reduced the accuracy of the actual diagnosis. Differential dementia diagnoses are difficult since many patients have a mixed pathology making a diagnosis of 1 specific type of dementia difficult.[36] Another ongoing problem is under-diagnosis of dementia in primary care which has been reported to be more than 50% in the United Kingdom.[37] The under-diagnosis could have biased our analysis, but would eventually drive the results toward the null hypothesis.

In conclusion, we assessed some of the most widely studied risk factors for dementia in late-life. We found the factors associated with an increased risk of all-cause dementia were physical inactivity, depression, and impaired fasting glucose. A protective relationship was found for overweight (BMI 25–29.9), as compared with normal weight women. These risk factors are all considered modifiable and therefore provide further evidence that prevention strategies could be a way to counteract the otherwise poor future prospects for dementia in the ageing population.

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5

Metabolic Syndrome, Insulin Resistance and Cognitive Dysfunction

Metabolic Syndrome, Insulin Resistance and Cognitive Dysfunction: Does your metabolic profile affect your brain?

Jesper S Neergaard[a,b,c], MSc, Katrine Dragsbæk[a,b], MSc, Claus Christiansen[a], MD, DMSc, Henning B Nielsen[c], MD, DMSc, Susanne Brix[b], PhD, Morten A Karsdal[a], PhD, Kim Henriksen[a], PhD.

Affiliations:

[a]Nordic Bioscience A/S, Herlev, Denmark

[b]DTU Bioengineering, Technical University of Denmark, Kgs. Lyngby, Denmark

[c]ProScion A/S, Herlev, Denmark

Running title: Insulin Resistance and Cognitive Dysfunction

Corresponding author:

Jesper Skov Neergaard, Nordic Bioscience A/S, Herlev, Denmark

Phone: +45 4452 5252, E-mail: jsn@nordicbio.com

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Abstract

Dementia and type 2 diabetes are both characterized by long prodromal phases challenging the study of potential risk factors and their temporal relation. The progressive relation between metabolic syndrome, insulin resistance, and dementia has recently been questioned, wherefore the aim of this study was to assess the potential association between these precursors of type 2 diabetes and cognitive dysfunction. Using data from the Prospective Epidemiological Risk Factor study (n=2,103), a prospective study of elderly women in Demark, we found that impaired fasting plasma glucose was associated with 44% (9%-91%) larger probability of developing cognitive dysfunction. In addition subjects above the HOMA-IR threshold (HOMA-IR > 2.6) had 47% (9%-99%) larger odds of cognitive dysfunction. The associations could indicate that a significant proportion of dementia cases in women is likely to be preventable by effective prevention and control of the insulin homeostasis.

The sedentary western life-style has led to an epidemic-like increase in prevalence of obesity that is closely linked to occurrence of type 2 diabetes (1,2). Also the prevalence of cognitive dysfunction and dementia is increasing and epidemiological studies suggest an association between type 2 diabetes and increased risk of dementia and cognitive dysfunction (3). With metabolic syndrome (MetS) considered a precursor of type 2 diabetes (4) and central obesity and insulin resistance (IR) being recognized as important causative factors in the pathogenesis of MetS (5), a precursor state for dementia may be developed over several years.

The long prodromal phases characterizing dementia and type 2 diabetes challenges the study of potential risk factors and their temporal relation (6,7) and in studies with short follow-up, putative relationships may be unreliable. Thus, reported associations between type 2 diabetes, MetS, and cognitive dysfunction are somewhat contrary. Until recently the brain was considered an insulin insensitive organ, it has however now been accepted that insulin, partly of peripheral origin, acts through its own receptors in the brain controlling cognition and memory(8). Thus it may be that IR is a condition affecting both peripheral and central insulin receptors with cerebral IR being part of a preclinical state of Alzheimer's disease(9). Importantly, the temporal relation between MetS, IR, and cognitive dysfunction/dementia has recently been questioned (10,11). This prompted us to conduct the current study in which data obtained as part of The Prospective Epidemiological Risk Factor (PERF) study, a prospective study of Danish postmenopausal women (12), underwent an evaluation with the aim to study the hypothesis that there is a temporal relation between MetS and IR and cognitive dysfunction. Data from PERF were used to evaluate whether there is an association between the MetS or IR and cognitive impairment at a follow-up 15 years later including only subjects without signs of cognitive dysfunction at the baseline examination (n = 1759).

Research Design and Methods

The Prospective Epidemiological Risk Factor Study

The Prospective Epidemiological Risk Factor (PERF) Study, an observational, prospective cohort study of Danish postmenopausal women, was designed with the purpose to obtain knowledge of age-related diseases in postmenopausal women. The baseline examination (PERF I) took place between 1999 and 2001 (n=5,855) and over fourteen months (from September 2013) 2,103 participants were included in a follow-up (PERF II) as described previously (12). The studies were carried out in accordance with ICH-GCP with study protocol approval from The Research Ethics Committee of Copenhagen County. Written informed consent was obtained from all subjects prior to any study related procedures.

Study population

This study was based on all subjects that completed the follow-up examination, PERF II (n = 2,103) and from this population we identified the analytical sample as outlined in figure 1.

(figure 1 here)

The study population included all subjects with valid cognitive tests at baseline and follow-up. Exclusion criteria were cognitive dysfunction at baseline and missing data on any of the confounders included in the analysis. This qualified 1,759 subjects for the analysis.

Cognitive dysfunction

Two short cognitive screening tests were applied to assess cognitive function at baseline and follow-up. The Short Blessed Test (SBT) is a six-item test assessing orientation, concentration, and memory. The score ranges from 0 to 28, with lower scores indicating better performance. A threshold of ≥10 was previously identified as cognitive impairment consistent with dementia (13). The category fluency test with animal naming (CFT) is a measure of verbal fluency where the subjects should name as many animals as possible in 60 seconds. Higher scores indicate better performance and the recommended threshold for dementia is ≤14 (14).

Metabolic Syndrome at baseline

MetS was defined using a modified version of the definition recommended by the International Diabetes Federation(15). Beside the entrance criteria of central obesity subjects should present two or more of the following risk factors: Increased triglycerides (>1.7 mmol/L), lowered level of HDL cholesterol (<1.29mmol/L), an increase in fasting plasma glucose (>5.6 mmol/L) or previously diagnosed type 2 diabetes, hypertension (systolic pressure above 130 mmHg or diastolic pressure higher than 85 mmHg or existing treatment of hypertension) to qualify for MetS. A direct measure of waist circumference was not obtained at baseline and therefore, the entrance criteria of central obesity was only defined by a BMI above 30 kg/m2 and as specific hyperlipidemia treatment was not part of the baseline questionnaire, we are unable to determine

whether participants were on specific lipid-lowering medication. Subjects without MetS were divided into three groups: *i)* subjects having a BMI >30kg/m2, and only one additional risk factor; *ii)* subjects presenting BMI <30kg/m^2 but with 1-4 risk factors for MetS; and *iii)* subjects without any risk factors for MetS. This group was used as the reference group in the regression analysis.

Insulin resistance and Glycosylated hemoglobin

HOMA-IR index was used to assess the degree of IR (16). The HOMA-IR index was calculated by fasting levels of plasma glucose multiplied by the concentration of insulin divided by the constant 22.5. Fasting plasma glucose was measured directly after collection in both PERF I and II, using a Vitros 250 slide cartridge with no reagent system from Ortho Clinical, in PERF I, and an enzymatic measurement method using the Avida 1800, from Siemens, in PERF II. Insulin levels at PERF I and PERF II was measured in thawed samples from the PERF biobank (stored at -80°C) on a Cobas e411 analyser from Roche. The level of Glycosylated hemoglobin (HbA1c) was measured using the Avida 1800 from Siemens and used to determine the outcome at follow-up. Blood samples were collected fasting in the morning.

Statistical analysis

Statistical analysis was conducted using R version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria). Spearman's Rank-Order Correlation was used to measure the association between scores of the two cognitive tests. By use of the *glm* function, logistic regression assessed the association between risk factors for the MetS, metabolic profiles and cognitive dysfunction. Three separate multivariable analyses were completed. In all analyses, baseline age and baseline cognitive performance were included as continuous variable and education level (primary school/high school/university), smoking history (never/former/current), alcohol consumption (none/<10.5 alcohol units per week/10.5-21 alcohol units per week/>21 alcohol units per week) and physical activity (Inactive/1 time per Week/2 times per Week/3+ times per week) and current use of hormone replacement therapy (yes/no) as categorical covariates.

We first tested each of the single risk factors comprising the MetS. The variables were dichotomized as described under "*Metabolic Syndrome at baseline"* above. Using the dichotomized variables we then studied how metabolic profiles at baseline were associated with cognitive dysfunction. First, we used the modified definition of MetS followed by the cumulative sum of MetS risk factors, ranging from zero to five, then we assessed the association between IR and risk of cognitive dysfunction. The baseline HOMA-IR index was used as continuous variable and further dichotomized at 2.6, where subjects above the threshold was considered insulin resistant. The outcome variables used were *i)* cognitive dysfunction on the SBT (SBT≥10), *ii)* cognitive dysfunction on the CFT (CFT≤14), and *iii)* cognitive dysfunction on both SBT and CFT (SBT≥10 and CFT≤14).

The Hosmer-Lemeshow test was used to test the goodness of fit for the logistic regression models.

Results

Of the 1,759 subjects included in the analysis, 136 had cognitive dysfunction according to the SBT, while 326 were classified with cognitive dysfunction when it was determined by CFT. A total of 80 subjects showed signs of cognitive dysfunction on both tests.

Characteristics of the study population

The baseline characteristics of the study population is shown in table 1. All subjects were on average 68 years old at baseline, with the non-impaired group as the youngest and the group of subjects with impaired cognition on both tests as the oldest.

(table 1 here)

There was a negative correlation between scores in the SBT and the CFT (rho = -0.294 [-0,336 to -0,250], p <0.0001).

The association between Metabolic Syndrome, Insulin resistance and cognitive dysfunction

Table 2 shows the association between metabolic risk factors, MetS, IR and cognitive dysfunction at follow-up. Fasting plasma glucose was associated with impairment in CFT suggesting that hyperglycemia increases the risk for development of cognitive dysfunction with 44% (OR 1.44, 95% CI 1.09-1.91). Having from one to four metabolic risk factors did not significantly alter the risk of cognitive dysfunction at follow-up when compared to subjects with no risk factors. In subjects with the worst metabolic profile, holding all five risk factors for MetS, the risk for cognitive dysfunction on verbal fluency was three times higher (OR 3.09, 95% CI 1.09-8.69) as compared to subjects who did not present any of the MetS risk factors. MetS was however not associated with increased risk of cognitive dysfunction at follow-up.

(table 2 here)

IR was associated with an increased risk of cognitive dysfunction, calculated both as CFT and a combination of the SBT and the CFT (Table 2). The risk of cognitive dysfunction increased between 8-10% for every unit increase on the HOMA-IR index scale and when dichotomized, subjects above the threshold of 2.6 had a 47% higher risk of cognitive dysfunction on verbal fluency (OR 1.47, 95% CI 1.09-1.99) as compared to subjects below the HOMA-IR threshold.

Discussion

In the present study we assessed the temporal relation between biomarkers and precursors of type 2 diabetes and cognitive dysfunction and specifically we evaluated whether MetS and IR are associated with development of cognitive dysfunction. Based on data with a follow-up period of up to 15 years it is demonstrated that *i)* subjects with impaired fasting plasma glucose have larger odds of developing cognitive dysfunction and *ii)* subjects with IR as determined by the HOMA-IR index have higher probability of developing cognitive dysfunction. While fasting plasma glucose were specifically associated with dysfunction on the verbal fluency test, IR seemed to result in more global cognitive dysfunction as determined by a combination of two short cognitive screening tests. The third important finding is that subjects with a poor metabolic profile, reflected by the presence of several metabolic and cardiovascular risk factors, have a 3- to 4-fold larger odds of developing cognitive dysfunction than subjects with an ideal metabolic profile. Overall the data suggest that IR is a cause rather than a consequence of cognitive dysfunction.

Fasting plasma glucose was the single metabolic risk factor that was most strongly associated with cognitive dysfunction. With cognitive function assessed by the CFT, subjects with impaired fasting plasma glucose levels had a 44% (9%-91%) larger odds of cognitive dysfunction as compared to normoglycemic subjects. While presence of MetS in itself does not seem to provoke an elevated risk of cognitive dysfunction, subjects with a poor metabolic profile have a three to four time's larger odds of developing cognitive dysfunction when compared to subjects with an ideal metabolic profile. The Framingham cohort have recently shown that subjects with ideal cardiovascular health, determined from a 7-point scale proposed by the American Heart Association, are at lower risk of dementia, cognitive decline and brain atrophy(17). Out of the seven risk factors defining an ideal cardiovascular health profile, four is identical or at least very similar to those defining the MetS, suggesting that cardiovascular and metabolic health is closely linked to brain health.

Peripheral IR has been shown to alter the transport of insulin through the blood-brain barrier. The insulin transport is reduced by peripheral hyperinsulinemia (18), which can directly contribute to cognitive impairment and promote AD pathology(19,20). It has also recently been shown that IR predicts worse memory performance through a reduction in regional cerebral glucose metabolism (21), supporting IR being a causal risk factor for development of cognitive dysfunction. While the study design does not allow for causal conclusions, the data presented here can be taken to indicate a temporal relation between IR and cognitive dysfunction. However, we cannot rule out the possibility that dementia or cognitive dysfunction leads to a diabetic phenotype and that a disturbance in insulin homeostasis, as a secondary process, may accelerate certain dementia pathologies (22). IR may be a shared underlying pathological mechanism, since it is part of the prodromal phase of both type 2 diabetes and dementia. Interestingly amyloid formation is a

pathological hallmark of both type 2 diabetes and AD: islet amyloid polypeptide is found in the pancreas of subjects with type 2 diabetes and β-amyloid is in the brain of subjects with AD (23). A recent study even suggest that pancreatic derived amyloid may enter the brain and exacerbate the deposition of β-amyloid through cross-seeding (24).

There are previous studies indicating an association between sleep disturbances and dementia (25). Mechanisms underlying the association are many, and IR is speculated to play an important role, however the causal link has not been elucidated. The menopausal transition is associated with sleep disturbances, which are also found to increase the risk of type 2 diabetes (26,27). As we observed a link between IR and cognitive dysfunction, it could indicate that IR is an intermediate mechanism for the causal association between sleep disturbances and cognitive dysfunction. We can however not address this in the current study as we did not collect information on sleep disturbances and sleep patterns at baseline.

The small, albeit significant, correlation between the two tests was expected and indicate that the two tests are not equivalent. This was reflected in the observed domain-specific effect of fasting plasma glucose and IR on cognition specifically related to verbal fluency. A similar domain-specific effect on verbal fluency has previously been found in two cross-sectional studies (28,29). One of the studies found that the effect of IR on cognition was modulated by gender, indicating that IR was associated with poor performance on verbal fluency only in women. Verbal fluency performance is functionally linked to the frontal and temporal lobe areas. These brain areas rich in insulin receptors, are found to be associated with memory function(28,30). There are several neuropathological conditions that affect memory-related areas in the brain, with AD being one of them. A structural alteration of semantic networks located in the frontal and temporal lobe areas has been found to be characteristic for AD even in the early stages of AD (31,32).

The concept of precision medicine is emerging in relation to prevention and treatment of AD (33) and the abundant evidence of various AD phenotypes, the metabolic phenotype being one, suggests that it is extremely relevant in this field. A recent meta-analysis indicate that insulin sensitizer drugs, like metformin and thiazolinediones, might be useful in the prevention of dementia in diabetic patients (34). Whether there is a direct mechanistic link is still controversial, but evidence from rat studies has shown that the glucagon-like peptide 1 analog liraglutide, another insulin sensitizer, interacts directly with processes leading to amyloid plaques and neurofibrillary tangles, the two pathological hallmarks of AD (35,36). Moreover, clinical trials have shown promising effects of intranasal insulin in subjects with AD and its prodrome, mild cognitive impairment (37,38) and also on spatial memory in young men (39).

The analysis was restricted to subjects attending the follow-up examination, therefore selection bias may affect the internal validity and question the generalizability of our results as it is well-known that cognitive dysfunction and dementia affect attrition. We have previously assessed the similarities between follow-up participants and follow-up non-participants on a cohort level, and found that the two populations are very similar (12). This should strengthen the internal validity. Further, we based our determination of cognitive dysfunction on two short cognitive screening tools at the follow-up visit, therefore we cannot not rule out the possibility that cognitive dysfunction in the current study may be caused by reversible conditions and thereby potentially result in misclassification. The diagnostic accuracy of the two tests in relation to dementia is excellent (40–43). They have even been shown to outperform more comprehensive tests like the Mini Mental State Examination in the identification of milder levels of impairment (44,45). In the absence of a comprehensive diagnostic workup with a complete neuropsychological test battery, this evidence support the use of these simple tests.

Another limitation is the lack of repeated measurement of glucose, insulin and cognition throughout the follow-up period as it would allow for a better assessment of the mutual trajectories and also resulted in a more accurate determination of the onset of cognitive dysfunction. Given the previously reported interconnection between genetic and metabolic risk factors, the lack of genetic risk factors in our studies is a limitation that could result in unmeasured confounding. For example it has been suggested that the insulin metabolism may differ between Apolipoprotein E epsilon 4 allele carriers and non-carriers (46).

Conclusion

The precursors of type 2 diabetes; impaired fasting plasma glucose and IR, are associated with increased risk of developing cognitive dysfunction in elder women. Moreover, subjects with a poor metabolic profile are more likely to develop cognitive dysfunction than subjects with an ideal metabolic profile. If the observed association between metabolic risk factors and cognitive dysfunction is truly causal it could suggest that a significant proportion of dementia cases in women may be preventable by effective control of insulin homeostasis.

Acknowledgments

KH is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis

Author contributions

JSN: wrote the manuscript, performed the literature search, data and statistical analysis, data interpretation. KD: reviewed and revised the manuscript, supported data interpretation and statistical analysis CC: contributed to the study design, acquired data and gave scientific advice. MAK: reviewed, and revised the manuscript including data interpretation and scientific advice. HBN and SB: reviewed and revised the manuscript KH: reviewed and revised the manuscript, supported data interpretation and gave scientific advice. All authors approved the final version of the manuscript.

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Conflicts of Interest

JSN, KD and SB reports no disclosures. CC serves as board member and stock owner in Nordic Bioscience A/S. HBN are full-time employee of ProScion A/S. MAK and KH are full-time employees of and hold stocks in Nordic Bioscience A/S.

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Tables

Table 1: Baseline characteristics of the study population. Numbers are shown as absolute numbers with percentile in brackets for categorical variables. For numerical variables the mean ± standard deviation (SD) are shown.

Variable	Non impaired n = 1377	SBT ≥ 10 n = 136	CFT ≤ 14 n = 326	SBT ≥ 10 CFT ≤ 14 n = 80
Demographics				
Age (years)	66.9 ± 5.6	70.6 ± 6.5	70.5 ± 5.8	72.4 ± 5.7
Education: Primary school, n (%)	903 (65.6)	96 (70.6)	225 (69.0)	56 (70.0)
High School, n (%)	332 (24.1)	26 (19.1)	77 (23.6)	17 (21.2)
University, n (%)	142 (10.3)	14 (8.1)	24 (7.4)	7 (8.8)
Lifestyle				
BMI (kg/m2)	26.0 ± 4.0	26.3 ± 4.8	26.5 ± 4.4	26.3 ± 4.3
<18,5, n (%)	19 (1.2)	1 (0.7)	2 (0.6)	0
18,5-24,9, n (%)	686 (42.3)	63 (46.3)	133 (40.8)	36 (45.0)
25,0-29,9, n (%)	653 (40.2)	46 (33.8)	127 (39.0)	28 (35.0)
≥30,0, n (%)	265 (16.3)	26 (19.1)	64 (19.6)	16 (20.0)
Smoking History: Never, n (%)	723 (52.5)	68 (50.0)	167 (51.2)	45 (56.2)
Former, n (%)	403 (29.3)	41 (30.1)	89 (27.3)	23 (28.7)
Current, n (%)	251 (18.2)	27 (19.9)	70 (21.5)	12 (15.0)
Alcohol: None, n (%)	512 (37.2)	66 (48.5)	148 (45.4)	36 (45.0)
<10.5 alcohol units/week, n (%)	312 (22.7)	22 (16.2)	61 (18.7)	15 (18.8)
10.5–21 alcohol units/week, n (%)	423 (30.7)	38 (27.9)	89 (27.3)	22 (27.5)
>21 alcohol units/week, n (%)	130 (9.4)	10 (7.4)	28 (8.6)	7 (8.8)
Physical activity: Inactive, n (%)	306 (22.2)	40 (29.4)	103 (31.6)	22 (27.5)
1 time /week, n (%)	310 (22.5)	29 (21.3)	54 (16.6)	17 (21.2)
2 times/week, n (%)	204 (14.8)	18 (13.2)	48 (14.7)	11 (13.8)
3+ times/week, n (%)	557 (40.5)	49 (36.0)	697 (37.1)	30 (37.5)
Metabolic and Vascular factors				
Systolic Blood Pressure (mmHg)	145.5 ± 23.1	148.9 ± 23.7	148.8 ± 23.2	150.2 ± 23.9
Diastolic Blood Pressure (mmHg)	81.9 ± 10.5	82.0 ± 10.5	82.0 ± 11.0	81.5 ± 10.8
Fasting Plasma Glucose (mmol/L)	5.4 ± 1.0	5.6 ± 1.5	5.6 ± 1.1	5.8 ± 1.8
Insulin (mmol/L)	54.9 ± 34.6	58.7 ± 44.5	60.9 ± 38.8	61.5 ± 42.1
HOMA-IR	2.0 ± 1.5	2.4 ± 3.6	2.3 ± 2.6	2.6 ± 4.3
High density lipoprotein (mmol/L)	1.7 ± 0.4	1.7 ± 0.4	1.8 ± 0.5	1.8 ± 0.4
Triglycerides (mmol/L)	1.3 ± 0.6	1.4 ± 0.7	1.4 ± 0.7	1.4 ± 0.7
Cognitive performance				
Short Blessed Test	1.3 ± 1.9	2.4 ± 2.4	1.7 ± 2.1	2.4 ± 2.3
Category Fluency Test	24.3 ± 5.2	21.5 ± 4.5	20.6 ± 4.2	20.8 ± 4.4

Table 2: Association between Metabolic Syndrome, Insulin resistance and cognitive dysfunction

	Cognitive status at follow up					
	SBT ≥10		CFT ≤14		SBT ≥10 & CFT≤14	
Predictor variables	OR*	95% CI	OR*	95% CI	OR*	95% CI
Individual Component of the MetS						
Body Mass Index (>30kg/m2)	1.22	0.76 - 1.94	1.24	0.88 - 1.74	1.26	0.70 - 2.30
Elevated Blood Pressure	0.88	0.56 - 1.38	1.07	0.76 - 1.50	0.68	0.38 - 1.23
Impaired Fasting Plasma Glucose	1.12	0.76 - 1.64	**1.44**	**1.09 - 1.91**	1.56	0.96 - 2.52
Low High Density Lipoprotein	1.01	0.59 - 1.74	1.19	0.81 - 1.74	0.99	0.47 - 2.09
Elevated Triglycerides	1.25	0.81 - 1.91	0.98	0.71 - 1.36	1.09	0.61 - 1.95
Cumulative sum of risk factors for MetS						
0 risk factors			reference			
1 "	0.72	0.40 - 1.27	1.02	0.65 - 1.59	0.72	0.34 - 1.56
2 "	0.64	0.35 - 1.19	1.06	0.66 - 1.69	0.60	0.27 - 1.38
3 "	1.18	0.61 - 2.27	1.19	0.70 - 2.03	1.02	0.41 - 2.52
4 "	0.59	0.22 - 1.60	1.39	0.71 - 2.71	0.66	0.19 - 2.33
5 "	2.56	0.75 - 8.79	**3.07**	**1.09 - 8.69**	**4.35**	**1.02 - 18.6**
Metabolic Syndrome						
No MetS			reference			
Risk factors for MetS with BMI < 30 kg/m2	0.98	0.65 - 1.49	1.08	0.80 - 1.46	0.94	0.55 - 1.61
BMI >30kg/m2 and < 2 risk factors	1.11	0.53 - 2.33	1.30	0.77 - 2.19	1.61	0.69 - 3.77
Metabolic Syndrome	1.28	0.71 - 2.29	1.30	0.82 - 1.94	1.18	0.55 - 2.55
Insulin Resistance (HOMA-IR)						
Dichotomized (HOMA-IR > 2.6)	0.98	0.64 - 1.52	**1.47**	**1.09 - 1.99**	1.33	0.77 - 2.27
Continuous (per unit increase)	1.05	0.98 - 1.13	**1.08**	**1.01 - 1.16**	**1.10**	**1.01 - 1.19**

*Odds ratios were adjusted for Age at Baseline, Smoking history, Alcohol Consumption, Physical Activity, Education and Hormone replacement therapy

Figures

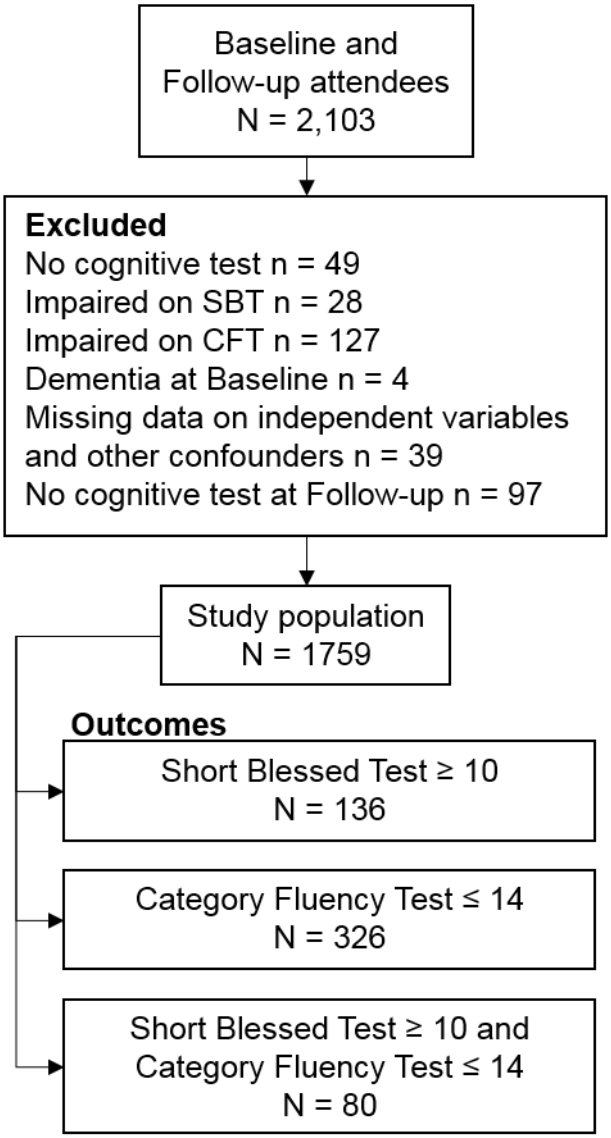

Figure 1: Flowchart for the identification of the analytical sample. Each outcome was determined independent of the other outcomes. SBT: Short Blessed Test, CFT: Category Fluency test.

6

Objective Cognitive Impairment and Progression to Dementia in Women

Original Research

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Objective Cognitive Impairment and Progression to Dementia in Women: The Prospective Epidemiological Risk Factor Study

J. Skov Neergaard[1,2], K. Dragsbæk[1,2], C. Christiansen[1], M. Asser Karsdal[1], S. Brix[2], K. Henriksen[1]

1. Nordic Bioscience A/S, Herlev, Denmark; 2. DTU Bioengineering, Technical University of Denmark, Kgs. Lyngby, Denmark

Corresponding Author: Jesper Skov Neergaard, Nordic Bioscience A/S, DK-2730 Herlev, Denmark, jsn@nordicbio.com, Phone: +45 4452 5252; Fax: +45 4454 7765

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Abstract

BACKGROUND: Identification of subjects with a progressive disease phenotype is an urgent need in the pharmaceutical industry where most of the recent clinical trials in Alzheimer's disease have failed.

OBJECTIVES: The objective of this study was to identify subgroups of individuals with objective cognitive impairment (OCI), who were most likely to progress to dementia and to identify the risk factors associated with progression.

DESIGN: Prospective cohort study.

SETTING: Population-based.

PARTICIPANTS: 5,380 elderly women from Denmark.

MEASUREMENTS: The Short Blessed Test and a category fluency test with animal naming, was used to assess cognitive function, and to classify them into different groups of OCI.

RESULTS: OCI was identified in 852 subjects at baseline. The risk of dementia was elevated for OCI subjects as compared to subjects with normal cognition (HR 1.46[1.19-1.79]). The courses of OCI were studied in a sub-cohort who completed the cognitive assessment at both the baseline and the follow-up visit (n = 1,933). Of these subjects 203 had OCI at baseline. The multi-domain subtypes of OCI were associated with progressive OCI. Subjects most likely to progress were older, physically inactive, had a higher level of total cholesterol (>6.5 mmol/L) and had a history of depression as compared to subjects with a non-progressive course of OCI.

CONCLUSIONS: In this cohort we identified a risk profile associated with progression from OCI in older women. The degree of impairment at baseline was an important predictor of conversion to dementia, additionally several modifiable risk factors were associated with progression.

Key words: Dementia, Cohort studies, Mild Cognitive Impairment, Cognitive Impairments.

Introduction

Mild Cognitive Impairment (MCI) has become the most widely used term to describe the subtle cognitive changes in the prodromal phases of dementia. The causes of MCI are not yet completely understood, it is however well-known that MCI increases the risk of later developing dementia, although some people with MCI never progress or even return to a normal cognitive state (1, 2). MCI is a heterogeneous condition (3, 4) and previous research suggests that the domains involved in MCI are not uniform across different subtypes (5).

Identification of subpopulations with pre-symptomatic disease is an urgent need in the pharmaceutical industry where most of the recent clinical trials in AD have failed, most likely due to patient selection difficulties (6). Identification of high-risk subjects with a progressive disease phenotype would likely increase the rate of success for disease-modifying interventions. In the absence of reliable biomarkers, risk profiles remain one of the best alternatives in identifying subjects with the highest likelihood of progression, underlining the need for identification of risks factors for progression.

In the current study, we used two short cognitive screening tests, namely the Short Blessed Test (SBT) and a category fluency test with animal naming (CFT), to assess cognitive function in 5,380 older women from the PERF study (7, 8). Based on their objective cognitive performance subjects were grouped in four subgroups. A total of 852 women were classified with objective cognitive impairment (OCI) at baseline. The study aimed to investigate the risk of progression to dementia from the four subtypes of OCI. Further, we assessed the risk profile for progression by studying the cognitive courses in a subgroup of subjects who attended a follow-up visit 15 years after baseline.

Materials and Methods

Study population

The PERF study was an observational, prospective study originally designed to study age-related diseases in women. The baseline examination took place in 1999-2001 (n=5,855) with a follow-up visit in 2013-2014 (n=2,103). Except for being women and postmenopausal, there were no other in/exclusion criteria at the time of enrolment. The baseline and follow-up visits comprised a physical examination, blood sampling and a self-reported questionnaire compiling information on medical history, medication, smoking status, alcohol intake, and physical activity. The cohort has been described in details

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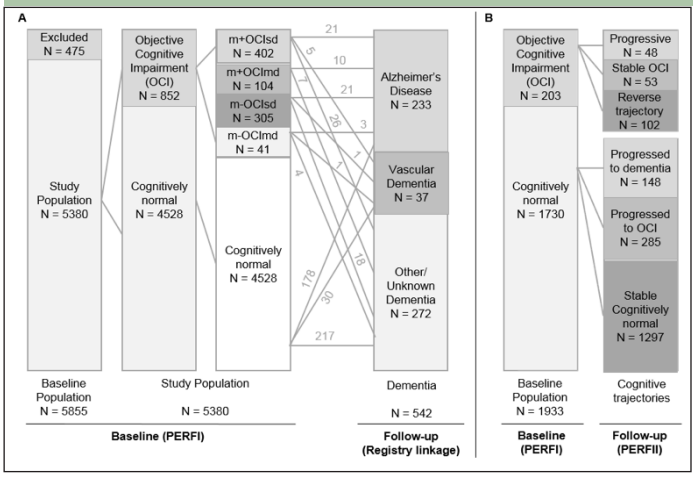
previously (9).

Of the baseline population (n=5,855), a total of 257 subjects were excluded since they did not undergo neuropsychological testing at the baseline examination. A further 206 subjects were excluded based on their cognitive performance at baseline, indicating cognitive impairment consistent with dementia (SBT ≥10). Lastly 12 subjects were excluded from the analysis due to a dementia diagnosis derived from the registry prior to study enrolment (Figure 1A).

Figure 1. Overview from baseline to follow-up



A: Flow diagram of OCI subtypes and progression to dementia. B: Overview of courses for progression from baseline to follow-up.

In the study of cognitive courses we identified all subjects with valid cognitive tests at both study visits who were dementia free at baseline (n= 1,933) (Figure 1B).

The studies were carried out in accordance with ICH-GCP with study protocol approval from The Research Ethics Committee of the Copenhagen County. Written informed consent was obtained from all subjects prior to study enrolment.

Cognitive Screening Tests

The SBT is a six-item test assessing orientation, concentration, and memory. Scores range from 0 to 28, with lower scores indicating better performance (7). A threshold of ≥10 was previously identified as cognitive impairment consistent with dementia (10). The CFT measures semantic fluency and executive functions; in this test subjects are asked to name as many animals as possible in 60 seconds. Higher scores indicate better performance (8). Both tests were oral, and administered by the investigator.

Determination of dementia outcome

Follow-up information on dementia status was retrieved from the National Danish Patient Registry and the Danish Register of Causes of Death using a unique personal identification number for each subject. The follow-up started on the day of study enrolment and ended at occurrence of event (dementia diagnosis), death, assessment of cognitive function at follow-up, or on Dec 31th 2014 (retrieval of registry data), whichever came first. A total of 542 incident dementia cases were identified from the registries. Dementia diagnoses were classified according to The International Classification of Diseases, 10th revision (ICD10). The following codes were considered a dementia diagnosis: Other/Unspecified dementia (OD) [F02-F03 and R54, n=272], Alzheimer's disease (AD) [F00 and G30-G32, n=233] and Vascular dementia (VaD) [F01, n=37].

Definition of OCI subgroups

The International Working Group on Mild Cognitive Impairment outlined a consensus criteria for the categorization of MCI subtypes in 2004 (2). Differently from the consensus criteria, subjective memory complaints and activities of daily living were not used in the current study since this information was not collected at the baseline examination. Based solely on their objective cognitive performance, we therefore grouped subjects into four subtypes of OCI. We defined OCI as at least 1.5 SDs below age and education stratified norms derived from our cohort (Supplementary Table 1).

Subjects qualifying for OCI with impairment in only the memory domain of the SBT were classified as having single domain memory+ OCI (m+OCIsd), while subjects with impairment in memory and at least one additional domain was classified as having multi domain memory+ OCI (m+OCImd) . Impairment in a single domain other than memory was classified as memory- single domain OCI (m-OCIsd) and signs of impairment in more than one domain other than memory classified subjects with memory- multi domain OCI (m-OCImd). A total of 852 subjects was classified with OCI.

Courses of OCI

The courses of OCI were defined in a sub-cohort of subjects who completed the cognitive assessment at both the baseline and the follow-up examination (n = 1,933). The courses were based on the objective cognitive performance at the baseline and the follow-up visits. A total of 203 subjects with OCI at baseline attended the follow-up examination. The remaining 1,730 subjects had normal objective cognitive performance at baseline. The progressive courses from either normal cognition or OCI were defined by progression to dementia (reported at the follow-up) or cognitive impairment consistent with dementia at follow-up (SBT ≥10) (n=148 and n=48). A stable OCI group: OCI at both baseline and follow-up (n= 53) and likewise a stable group with normal cognitive performance at both baseline and follow-up (n=1,297). A

Table 1. Baseline characteristics of defined OCI subtypes and non-impaired group. Numbers are shown as absolute numbers with percentile in brackets for categorical variables. For numerical variables the mean and standard deviation (SD) are shown

		Subtypes of Mild Cognitive Impairment				
		Memory + OCI		Memory - OCI		
	Non-impaired N = 4528	single domain N = 402	multi domain N = 104	single domain N = 305	multi domain N = 41	P-value
Age (years)	70.4 ± 6.5	71.5 ± 6.1	71.6 ± 6.4	71.1 ± 6.2	73.7 ± 7.4	<0.001
Education, n (%)						
Primary school	3136 (69.3)	327 (81.5)	82 (78.8)	213 (69.8)	33 (80.5)	< 0.0001
High School	1047 (23.2)	53 (13.2)	18 (17.3)	62 (20.3)	6 (14.6)	
University	339 (7.5)	21 (5.2)	4 (3.8)	30 (9.8)	2 (4.9)	
BMI (kg/m^2)	26.1 ± 4.2	26.3 ± 4.3	26.6 ± 4.4	26.1 ± 4.3	27.7 ± 4.2	0.09
<18.5, n (%)	69 (1.5)	5 (1.3)	1 (1.0)	5 (1.7)	0	0.2
18.5-24.9, n (%)	1898 (42.0)	157 (39.4)	43 (41.3)	127 (42.1)	11 (26.8)	
25.0-29.9, n (%)	1797 (39.8)	166 (41.7)	36 (34.6)	126 (41.7)	16 (39.0)	
≥30.0, n (%)	754 (16.7)	70 (17.6)	24 (23.1)	44 (14.6)	14 (34.1)	
Smoking, n (%)						
Never	2149 (47.5)	196 (48.8)	50 (48.1)	140 (45.9)	19 (46.3)	0.6
Past	1391 (30.7)	111 (27.6)	32 (30.8)	89 (29.2)	9 (22.0)	
Current	984 (21.8)	95 (23.6)	22 (21.2)	76 (24.9)	13 (31.7)	
Alcohol consumption, n (%)						
Never	1870 (41.6)	210 (52.8)	60 (57.7)	147 (48.5)	16 (39.0)	0.0001
<10.5 alcohol units/week	1076 (23.9)	85 (21.4)	23 (22.1)	72 (19.5)	8 (19.5)	
10.5-21 alcohol units/week	1217 (27.1)	86 (21.6)	17 (16.3)	69 (22.8)	13 (31.7)	
>21 alcohol units/week	335 (7.4)	17 (4.5)	4 (3.8)	15 (5.0)	4 (9.8)	
Physical activity, n (%)						
None	1353 (29.9)	113 (28.2)	32 (30.8)	107 (35.1)	16 (39.0)	0.4
1 time/week	945 (20.9)	88 (21.9)	24 (23.1)	74 (24.3)	9 (22.0)	
2 times/week	596 (13.2)	57 (14.2)	13 (12.5)	39 (12.8)	5 (12.2)	
3+ times/week	1629 (36.0)	143 (35.7)	35 (33.7)	85 (36.0)	11 (26.8)	
Systolic Blood Pressure >160 mmHg or treated hypertension	1712 (37.9)	151 (37.7)	35 (34.3)	133 (43.6)	18 (43.6)	0.3
History of cerebral embolism/hemorrhage	132 (2.9)	12 (3.0)	4 (3.8)	9 (3.0)	2 (4.9)	0.9
Fasting Glucose						
Normal (<5.6 mmol/L)	2888 (64.2)	257 (64.6)	64 (62.1)	202 (66.9)	18 (43.9)	0.3
Impaired (5.6-6.9 mmol/L)	1378 (30.6)	120 (30.2)	34 (33.0)	88 (29.1)	20 (48.8)	
Hyperglycemic (≥7.0 mmol/L) or treated diabetes	230 (5.1)	21 (5.3)	5 (4.9)	12 (4.0)	3 (7.3)	
Total Cholesterol (>6.5 mmol/L) or treated hyperlipidemia	1963 (43.7)	176 (44.2)	48 (46.6)	129 (42.7)	22 (53.7)	0.7
History of depression	264 (5.8)	36 (9.0)	4 (3.8)	35 (11.5)	9 (22.0)	< 0.0001

reverse trajectory group comprised subjects with OCI at baseline who returned to normal cognitive performance at follow-up as determined from the norms in our cohort (n=103). The remaining 285 progressed from normal cognitive performance to OCI at the follow-up (Figure 1B).

Statistical Analysis

Statistical analysis was conducted using MedCalc Statistical Software version 16.8.4 (MedCalc Software bvba, Ostend, Belgium) and R version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria). The baseline characteristics in subjects with normal cognition and subjects with OCI were compared using a one-way analysis of variance (ANOVA) for quantitative variables and Chi-squared test for categorical variables (Table 1).

The prevalence was calculated as the fraction of subjects with OCI at baseline. The incidence of dementia was calculated by dividing the number of cases by the number of person-years at risk. The differences in all-cause and differential dementia incidence were assessed using pair-wise comparison of rates. Differences in time to dementia diagnosis was assessed using one-way ANOVA.

Age and educational adjusted cause-specific Cox proportional hazards regression were used to assess the association between subtypes of OCI and risk of all-cause dementia and its subtypes. Dementia-free mortality was included as a competing risk as outlined by Benichou and Gail (11). Follow-up time was used as time scale. Age was included as continuous variable, education level (primary school, high school or university) and subtypes of OCI as categorical variables. Risk of dementia was assessed jointly for all OCI cases, followed by a distinction between memory+ and memory- subtypes

Table 2. Association between Objective Cognitive Impairment (OCI) and Risk of Dementia

	All-cause Dementia		Alzheimer's Disease		Vascular Dementia		Other/Unspecified Dementia	
Groups of OCI	**HR***	**(95% CI)**	**HR***	**(95% CI)**	**HR***	**(95% CI)**	**HR***	**(95% CI)**
Cognitively normal	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
All OCI cases	1.46	(1.19-1.79)	1.65	(1.22-2.23)	1.23	(0.54-2.80)	1.33	(0.99-1.80)
Memory+ OCI	1.39	(1.08-1.80)	1.50	(1.03-2.21)	1.40	(0.54-3.63)	1.30	(0.90-1.89)
Single domain	1.31	(0.98-1.76)	1.27	(0.81-2.01)	1.77	(0.69-4.58)	1.29	(0.85-1.95)
Multiple domains	1.71	(1.05-2.78)	2.43	(1.28-4.59)	no cases		1.38	(0.65-2.93)
Memory- OCI	1.55	(1.15-2.09)	1.87	(1.22-2.87)	0.94	(0.22-3.93)	1.38	(0.89-2.14)
Single domain	1.47	(1.06-2.04)	1.85	(1.18-2.91)	0.54	(0.07-3.95)	1.29	(0.85-1.95)
Multiple domains	2.14	(1.06-4.31)	2.00	(0.64-6.26)	3.67	(0.50-27.0)	2.02	(0.75-5.45)

*Hazard ratios are adjusted for age and education

and finally split into the four subtypes. The group of subjects who did not meet the study specific definition of OCI was used as the reference group.

Logistic regression was used to assess the risk and corresponding risk factors for progression. Two separate analyses were conducted, one including the entire follow-up population and secondly an analysis including only those with OCI at baseline. The progressive course was compared with the non-progressive courses (stable and reverse trajectory). Age was included as continuous variable and the categorical covariates education level (primary school, high school and university), BMI (normal weight [≤25 kg/m2], overweight [>25<30 kg/m2] or obese [30+ kg/m2]), smoking (never, past or current), alcohol consumption (never, <10.5 alcohol units/week, 10.5-21 alcohol units/week or >21 alcohol units/week), physical activity (other than walking) (never, once weekly, twice weekly or three or more times per week), systolic blood pressure (>160 mmHg or treated hypertension), fasting glucose levels (≥7.0 mmol/L or treated diabetes) and total cholesterol levels (>6.5 mmol/L or treated hyperlipidaemia), history of depression (yes/no) and history of cerebral embolism/haemorrhage (yes/no) were included in the analyses. In relation to model diagnostics we used the Hosmer & Lemeshow test and the Nagelkerke pseudo R2 as goodness of fit measures.

Results

Baseline characteristics of the study population

Of the non-demented study population comprising 5,380 older women, 852 were categorized with OCI, while the remaining 4,528 had neuropsychological tests indicating normal cognition. All subtypes of OCI, excluding m-OCIsd, were significantly older as compared to the non-impaired group. The non-impaired group had generally higher levels of education (table 1). With regards to alcohol consumption the subtypes with memory involvement, in particular, had larger proportions of subjects that were abstinent and lower proportions of subjects that consumed larger amounts of alcohol. A history of depression was more frequent in those with m-OCI (table 1).

Prevalence of OCI and risk of progression to dementia

The overall prevalence of OCI was 16% (14.9-16.8). Among the subtypes of OCI the incidence of dementia was largest in the group with m-OCImd followed by m+OCImd, m+OCIsd and m-OCIsd (Supplementary Table 2). The incidence rates however had overlapping confidence intervals. For differential diagnoses, the incidence of AD was largest in the m+OCImd subgroup, while the incidences for OD and VaD were largest in subjects with m-OCImd, although still with overlapping confidence intervals (Supplementary Table 2). The fastest conversion rate (time to diagnosis) was found in subjects with m-OCImd. The average time to diagnosis of 5.1 years was significantly lower than the subtypes with single domain OCI only ($p = 0.05$).

Over the entire follow-up period of maximum 15 years (median 13.7 years), the risk of all-cause dementia was elevated for all OCI cases as compared to subjects with normal cognition (HR 1.46[1.19-1.79]). The association with differential diagnoses suggested that OCI was more strongly associated with AD (HR 1.65[1.22-2.23]) than with VaD or OD. For subtypes of OCI the maximum lengths of the follow-up period were 15 years (median 13.7 years), 15 years (median 13.7 years), 15 years (median 13.6 years), 14 years (median 12.0 years) for m+OCIsd, m+OCImd, m-OCIsd and m-OCImd, respectively. The association with all-cause dementia and AD was strongest for subjects with multi domain involvement, although the confidence intervals overlap. There was no association between OCI and its subtypes and VaD and neither with OD (table 2).

Courses of OCI and risk factors for progression

Subjects with OCI at baseline had distinct prognostic courses with 24% (48/203) having a progressive course,

26% (53/203) remaining stable and 50% (102/203) having a reverse trajectory from baseline to follow-up. The multi-domain subtypes of OCI, especially m+OCImd, were associated with progressive OCI. Several other risk factors were associated with progression. Age, hyperlipidaemia and history of depression were positively associated with progression, while physical activity (≥1 time/week) lowered the risk of progression from OCI (table 3).

The risk factors for progression in the entire follow-up population were age, abstinence from alcohol consumption, hyperlipidemia and history of depression.

Discussion

In this large prospective study we identified a high-risk progressive profile among subjects classified with OCI at baseline. We assessed which subtypes of OCI that were associated with progression to dementia and severe cognitive impairment. Subjects that were most likely to progress had m+OCImd, were older, physically inactive, higher level of total cholesterol and had a history of depression compared to subjects with a non-progressive course of OCI over the entire follow-up period.

Although the study design prevented us from using the core clinical criteria for MCI, the prevalence of OCI of 16% observed in the current study corresponds well with previous MCI studies, mostly within the range of 14-18% (12, 13). The incidence of dementia was largest in subjects with multi-domain involvement, while the time to diagnosis was shortest as compared to single domain OCI subtypes. These findings support the literature suggesting that the degree of impairment (single vs. multi-domain) at baseline is an important predictor of conversion to dementia (14-16). In the current study we found that OCI, and especially m+OCImd, were associated with higher risk of AD. Coherently, previous evidence suggests that subjects with amnestic MCI have increased risk for AD, while subjects with non-amnestic MCI may have a larger risk for other dementia types (17, 18).

Our finding that subjects with multi-domain OCI, the subtype with memory involvement in particular, were more likely to progress than those with single-domain OCI, corresponds well with previous findings, although these findings are primarily based in the MCI criteria by Petersen (16, 19, 20). In the current study, the risk profile associated with progression for women was characterized by advanced age, physical inactivity, higher total cholesterol levels and a history of depression. Likewise, researchers from the 3C study also found that age and depression predicted progression in women (21).

For alcohol consumption, results from the ILSA study show that subjects consuming up to 15 grams of alcohol per day (equivalent to 1.9 alcohol units in our study) had a lower rate of progression when compared to abstainers. Although the study uses different cut-off values for alcohol consumption the direction of the association is the same suggesting that light to moderate alcohol consumption is associated with lower risk of progression (22). The Nurses' Health Study found a similar association where moderate drinkers lowered their relative risk of impairment approximately 20%, as compared with abstainers (23).

Table 3. Risk Factors for Progression from Objective Cognitive Impairment (OCI)

	OCI only (n = 203) Progressive vs. Non-progressive	Entire population (n = 1933) Progressive vs. Non-progressive
Variable	**OR* (95% CI)**	**OR* (95% CI)**
Memory+ OCI single domain	2.21 (0.88-5.55)	
Memory+ OCI multi-domain	8.30 (1.79-38.5)	
Memory- OCI single domain	1 (reference)	
Memory- OCI multi-domain	5.18 (0.80-33.6)	
Age (per 5 years of ageing)	1.72 (1.15-2.57)	1.78 (1.56-2.04)
Education		
Primary school	1 (reference)	1 (reference)
High School	1.23 (0.41-3.72)	0.73 (0.49-1.09)
University	0.25 (0.05-1.32)	0.62 (0.35-1.10)
BMI		
Normal (<25)	1 (reference)	1 (reference)
Overweight (25.0-29.9)	0.60 (0.22-1.59)	0.81 (0.57-1.14)
Obese (≥30.0)	0.92 (0.30-2.85)	1.09 (0.71-1.69)
Smoking history		
Never	1 (reference)	1 (reference)
Past	1.63 (0.60-4.45)	1.10 (0.78-1.45)
Current	2.83 (0.90-8.94)	1.32 (0.87-1.99)
Alcohol Consumption		
None	2.07 (0.66-6.51)	1.60 (1.06-2.42)
Little (<10.5 units/week)	1 (reference)	1 (reference)
Moderate (10.5-21 units/week)	0.53 (0.13-2.23)	0.96 (0.60-1.52)
Heavy (>21 units/week)	0.18 (0.01-2.55)	0.84 (0.42-1.71)
Physical activity		
None	1 (reference)	1 (reference)
1 time/week	0.29 (0.09-0.94)	0.82 (0.53-1.25)
2 times/week	0.44 (0.13-1.55)	0.70 (0.43-1.15)
3+ times/week	0.28 (0.09-0.84)	0.77 (0.53-1.14)
Fasting Glucose		
<5.6 mmol/L	1 (reference)	1 (reference)
5.6-6.9 mmol/L	0.59 (0.23-1.53)	0.95 (0.68-1.32)
≥7.0 mmol/L or treated diabetes	1.60 (0.22-11.6)	1.12 (0.54-2.32)
Systolic Blood Pressure >160 mmHg or treated hypertension	0.91 (0.38-2.19)	1.27 (0.92-1.75)
Total Cholesterol (>6.5 mmol/L) or treated hyperlipidemia	4.82 (2.01-11. 5)	1.51 (1.11-2.04)
History of cerebral embolism/ hemorrhage (yes/no)	4.12 (0.24-70.8)	1.23 (0.54-2.78)
History of depression (yes/no)	4.72 (1.02-21.8)	1.85 (1.03-3.32)

*All odds ratios are mutually adjusted

We found a positive association between history of depression and progression. Similarly, a previous study found that subjects with MCI and depression had a two-fold increased risk of developing AD than those with MCI without depression (24). The Kungsholmen study however found no association between depressive symptoms and risk of dementia in a group of subjects with MCI at baseline (25). Our study is the first to report a positive association between higher total

cholesterol levels and a risk of progression from OCI to dementia. The 3C cohort found no association between hypercholesterolemia and progression from OCI to dementia. This conflicting evidence could potentially be explained by differences in baseline age and length of follow up, where women from the 3C study on average were nearly four years older and only followed for four years (21).

Limitations

First, the cohort only comprised women and therefore generalization cannot be made to men of similar ages. Further, our neuropsychological assessment could have been more comprehensive. Although the SBT is said to assess both orientation, concentration and memory and the CFT to assess verbal fluency, they are limited to a few cognitive domains and the study lacks assessment of perceptual or visual-spatial abilities and a more comprehensive assessment of memory. Nonetheless, the two tests have diagnostic accuracies in relation to dementia equivalent to the Mini Mental State Examination (26, 27) and the SBT has been proven to have very good predictive capacity since it is superior in the identification of milder levels of impairment (27). Further poor performance on delayed recall as assessed with memory question in the SBT indicates a high risk of progression to dementia and has also been used as diagnostic criteria for amnestic mild cognitive impairment in several large randomized clinical trials (12). All evidence that, in the absence of a complete neuropsychological test battery, supports the use of these simple tests.

Another limitation is the deviation from the core clinical MCI criteria. This was caused by the missing assessment of subjective memory complaints and activities of daily living. Unfortunately this makes the direct comparison with previous studies difficult since this could give raise concerns in relation to misclassification. In favour of valid comparisons is the previous evidence suggesting that the application of subjective memory complaints and activities of daily living may be questionable in population-based studies (28). Further, a previous study found that self-reported memory complaints did not predict a cognitive decline (29, 30). Subjective memory complaints have however also been reported to be associated with increased risk of cognitive impairment and dementia (31, 32). It is likely that the predictive value of subjective memory complains vary across different clinical settings. Finally, subjective memory complaints for subjects enrolled in population-based studies are normally elicited by standardized questions rather than being spontaneously reported, and their response may therefore vary in prognostic significance (33). It must however still be noted that the distinction between normal cognition and MCI are difficult and eventually rely on a clinical judgment which was not the case in the current study.

In the first part of our study we used registry-linkage to obtain information on incident dementia diagnoses. Registry-linkage is associated with very limited loss to follow up, however the validity of the diagnosis may be questioned. Similar registries are found in Sweden and Finland and studies from these countries indicate high validity and very good accuracy of the diagnoses, but underestimation is present (34, 35). In the analysis of risk factors for progression our analysis was restricted to subjects who attended the follow-up visit. This may question the generalizability of our findings as survivorship bias cannot be ruled out in studies of elderly women like the current study.

Conclusion

In this cohort we identified a risk profile associated with progression of OCI in women. The degree of impairment at baseline was an important predictor of conversion to dementia and several modifiable risk factors, including physical activity, alcohol consumption, total cholesterol levels and history of depression were associated with progression. The subgroups of OCI that were most likely to progress to all-cause dementia and dementia subtypes was multi-domain OCI.

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Supplement: Objective Cognitive Impairment and Progression to Dementia in Women: The Prospective Epidemiological Risk Factor Study

Supplementary Table 1. Population-based means (SD) and cut-off values of tests used for classification of Objective Cognitive Impairment

		Category Fluency			Short Blessed Test Total score			Short Blessed Test Memory question		
Age groups	**Level of education**	**Mean**	**SD**	**Cut-off**	**Mean**	**SD**	**Cut-off**	**Mean**	**SD**	**Cut-off**
50-60 (N = 264)	Primary School	22.6	5.2	≤14	1.8	2.1	>4	1.6	2.1	>4
	High School	24.9	5.6	≤16	0.7	1.3	>2	0.6	1.3	>2
	University	24.2	3.9	≤18	0.7	1.4	>2	0.6	1.1	>2
60-70 (N = 2185)	Primary School	22.2	5.5	≤13	1.9	2.3	>5	1.6	2.1	>4
	High School	23.4	5.6	≤14	1.4	2.0	>4	1.2	1.8	>3
	University	24.9	6.1	≤15	1.3	2.0	>4	1.1	1.8	>3
70-80 (N = 2498)	Primary School	20.3	5.4	≤12	2.3	2.5	>5	2.0	2.3	>5
	High School	21.8	5.7	≤13	1.6	2.0	>4	1.4	1.8	>4
	University	23.4	6.0	≤14	1.6	2.1	>4	1.4	2.0	>4
80+ (N = 433)	Primary School	18.9	5.2	≤11	2.7	2.6	>6	2.3	2.3	>5
	High School	19.9	5.0	≤12	1.9	2.3	>5	1.6	2.1	>4
	University	23.0	6.5	≤13	1.2	2.0	>4	1.2	1.9	>4

Supplementary Table 2. Progression to dementia in subtypes of Objective Cognitive Impairment. Differences in Incidence rates and average time to diagnosis were tested using one-way analysis of variance

	Subtypes of Objective Cognitive Impairment				
	m+OCI single domain N = 402	**m+OCI multi domain N = 104**	**m-OCI single domain N = 305**	**m-OCI multi domain N = 41**	**P-value**
Number of new cases	52	17	40	8	
Person years at risk	4731.7	1177.3	3482.7	409.8	
Dementia Incidence (per 1000 person years), (95 % CI)	11.0 (8.4-14.4)	14.4 (9.0-23.2)	11.5 (8.4-15.7)	19.5 (9.8-39.0)	ns
Differential incidence (per 1000 person years), (95 % CI)					
Alzheimer's Disease	4.4 (2.9-6.8)	8.5 (4.6-15.8)	6.0 (3.9-9.2)	7.3 (2.4-22.7)	ns
Vascular Dementia	1.1 (0.4-2.5)	no cases	0.3 (0.0-2.0)	2.4 (0.3-17.3)	ns
Other/Unspecified dementias	5.5 (3.7-8.1)	5.9 (2.8-12.5)	5.2 (3.3-8.2)	9.8 (3.7-26.0)	ns
Time to diagnosis (years), (95 % CI)	8.5 (7.4-9.5)	6.5 (4.3-8.7)	8.2 (7.1-9.4)	5.1 (2.4-7.9)	0.05

m+OCI: objective cognitive impairment with memory involvement, m-OCI: objective cognitive impairment without memory involvement

7

Two novel serum biomarkers measuring degradation of tau are associated with dementia

Two novel serum biomarkers measuring degradation of tau are associated with dementia: a prospective study

Jesper Skov Neergaard[a,b*], MSc, Katrine Dragsbæk[a,b], MSc, Claus Christiansen[a], MD, DMSc, Morten Asser Karsdal[a], PhD, Susanne Brix[b], PhD, Kim Henriksen[a], PhD

Affiliations:

[a]Nordic Bioscience A/S, Herlev, Denmark

[b]DTU Bioengineering, Technical University of Denmark, Kgs. Lyngby, Denmark

*Corresponding author:

Jesper Skov Neergaard, jsn@nordicbio.com

Nordic Bioscience A/S, DK-2730 Herlev, Denmark

Phone: +45 4452 5252. Fax: +45 4454 7765

Co-author emails: Katrine Dragsbæk (kdm@nordicbio.com); Claus Christiansen (cc@nordicbio.com); Morten Asser Karsdal (mk@nordicbio.com); Susanne Brix (sbp@bio.dtu.dk); Kim Henriksen (kh@nordicbio.com)

Abstract

Background: There is a need for non-invasive and reliable biomarkers to aid in early prognosis and diagnosis for neurodegenerative disorders. Truncated tau appears to be specifically related to disease pathology and recent studies have shown the presence and elevation of several truncated tau species in Cerebrospinal fluid (CSF) of subjects with Alzheimer's disease (AD). The aim of the current study was to assess the longitudinal associations between baseline levels of two novel serum biomarkers measuring truncated tau, Tau-A and Tau-C, and the risk of incident dementia and Alzheimer's disease in a large prospective cohort of nearly 6,000 elderly women.

Methods: Using solid phase competitive ELISA, two tau fragments were detected in serum of 5,309 elderly women from the Prospective Epidemiological Risk Factor (PERF) study. The PERF study was an observational, prospective study of Danish postmenopausal women. Subjects were followed with registry-linkage for a maximum of 15 years (median follow-up time 13.7 years).Cause-specific Cox regression was used to assess the utility of the biomarkers in relation to incident dementia and AD.

Results: High levels of Tau-A and Tau-C (above the median) were associated with lower risk of dementia and AD (Tau-A: Dementia HR [95% CI] = 0.85[0.70-1.04]; AD 0.71[0.52-0.98] and Tau-C: Dementia 0.84[0.70-1.00]; AD 0.78[0.60-1.03]). Tau-C gave a very modest increase in the AUC in a 5-year prediction horizon as compared to a reference model with age and education.

Conclusions: Measurement of tau in serum is feasible. The serological tau turnover profile may be related to the diagnosis and development of dementia and AD. The exact processing and profile in serum in relation to cognitive disorders remains to be further assessed to provide simple non-invasive serological tests to identify subjects with progressive cognitive disorders.

Keywords: Dementia, Alzheimer's disease, Serum Biomarkers, Prognosis

Background

The global burden of dementia is rising, with a new case registered every 3.2 seconds. Dementia is ranked as the 9th most burdensome disease for people aged 60 years and older, however the costs associated with dementia are enormous and place dementia as the most expensive disease in the United States. The reason for this increase in dementia prevalence and the following increased costs are mainly caused by the shifting epidemiological trend of increasing numbers of elder people, caused by low fertility rates and increasing longevity [1,2].

To counteract this dreary trend there is a need for better treatments. The success in pharmaceutical drug development has been greatly challenged due to the difficulties in detecting the disease at a stage allowing for intervention and thereby detecting efficacy. Consequently, there is a clear need for non-invasive and reliable biomarkers to aid in early diagnosis, prognosis and early efficacy assessment. Cerebrospinal fluid (CSF) biomarkers exist, and while they aid in diagnosis, their clinical utility is limited due to the invasive nature of the lumbar puncture.

Evidence suggests that tau is possibly *the* protein triggering and driving the process of cognitive decline and neuronal loss in Alzheimer's disease (AD)[3,4]. Besides AD, tau is known to be involved in the pathogenesis of several other neurodegenerative diseases referred to as tauopathies. The common denominator for these diseases is an alteration of the tau protein leading to the generation of neurotoxic tau aggregates known as neurofibrillary tangles (NFT). During this process the tau protein is known to undergo several different posttranslational modifications, where phosphorylation is among the most well studied. Several studies indicate that proteolytic processing of tau plays an important role in neurodegeneration and it has been suggested that caspase cleavage of tau may precede the hyper-phosphorylation, where especially caspase cleavage at Asp421 has been shown to initiate the cascade leading to tau aggregation[5–7].

Recently our research group developed two solid phase competitive ELISA assays detecting the caspase-generated fragment cleaved at Asp421 (Tau-C) and another detecting an ADAM10-generated fragment cleaved at Ala152 (Tau-A) of tau. These novel biomarkers have shown promising results in the initial biological validation: In ice hockey players suffering from mild traumatic brain injury, serum levels of Tau-C were significantly higher in post-concussion samples compared with preseason samples[8], confirming that tau processing and release into the circulation is associated with brain damage. Further, levels of Tau-A correlated with the duration of post-concussive symptoms, clearly indicating relevance to the neuronal damage[8]. In a smaller dementia cohort the tau fragments have been shown to be able to discriminate between AD and Mild Cognitive Impairment (MCI) which shows that the tau fragments can provide guidance on the differential diagnosis of dementia[9].

The aim of the current study was to assess the longitudinal associations between baseline levels of Tau-A and Tau-C and the risk of incident dementia and Alzheimer's disease in a large prospective cohort of 5,309 elderly women.

Methods

Study population

The Prospective Epidemiological Risk Factor (PERF) study was an observational, prospective study of Danish postmenopausal women. The cohort has been described in details elsewhere[10]. A total of 5,855 women aged 55–85 were enrolled in the study. Being woman and postmenopausal were the only inclusion criteria's at the time of enrolment. The baseline examination took place between 1999 and 2001 and comprised a questionnaire, physical examination and blood sampling at the study site. The study was carried out in accordance with ICH-GCP with study protocol approval from The Research Ethics Committee of Copenhagen County. Written informed consent was obtained from all subjects prior to any study related procedures.

Of the entire baseline population (n=5,855), a total of 206 subjects were excluded based on their cognitive performance at baseline, indicating cognitive impairment consistent with dementia (a Short Blessed Test score ≥10). Two hundred fifty-three subjects did not complete the cognitive testing at baseline and were also excluded in the current study. In addition, 12 subjects were excluded from the analysis due to a preexisting dementia diagnosis derived from the National Danish Patient Registry prior to study enrolment. Further 75 subjects were excluded since no serum samples was available for biomarker measurement. The analytical sample in the current study therefore constituted 5,309 subjects.

ELISA methodology

The neo-epitope fragments of tau were detected using solid phase competitive ELISA. Fragments were detected by mouse monoclonal antibodies raised against human tau. The antibodies detect an ADAM10-generated cleavage site at Ala152 (Tau-A) and the caspase-3-generated cleavage site at Asp421 (Tau-C). The monoclonal antibodies recognize a decamer sequence containing the cleavage site. Both assays have previously been described in details elsewhere[11,12]. The lower limit of quantification (LLOQ) for Tau-A was 29.4 ng/ml. For Tau-A 68% (n = 3,595) of samples were below the LLOQ. If their reported value were above the lower limit of detection (LLOD, n = 3,443) and their respective Intra-Assay Coefficients of Variability (CV) allowed for it (<15%) these samples were assigned their absolute value (n = 2,293). In total 1,150 samples in the range between LLOQ and LLOD were excluded from the main analysis due to an Intra-Assay CV ≥ 15%. A sensitivity analysis including these samples was performed as outlined in the statistical analysis section. The LLOD was 9.3 ng/ml. Samples measured below the LLOD were

assigned the LLOD value (n = 152). The LLOQ for Tau-C was determined as 8.6 ng/ml. For Tau-C, samples measured below the LLOQ were assigned the LLOQ value (n = 139). The LLOD for Tau-C was 0.8 ng/ml. The biomarker analysis were conducted at a College of American Pathology (CAP) certified central laboratory (Nordic Bioscience Laboratory). The staff at the central laboratory had no knowledge of the study participants.

Dementia diagnosis

Follow-up information on dementia status was retrieved from the National Danish Patient Registry and the National Danish Causes of Death Registry using a unique personal identification number for each subject. The follow-up started on the day of study enrollment and ended at the occurrence of an event (dementia diagnosis), death, or on the day of the retrieval of registry data (December 31th 2014), whichever came first. A total of 538 incident dementia cases were identified from the registries. Dementia diagnoses were classified according to The International Classification of Diseases, 10 th revision (ICD10). The following codes were considered a dementia diagnosis: F00-F04, G30-G32 and R54, while F00 and G30 was used to identify AD (n = 232).

Statistical analysis

Statistical analysis was conducted using R version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria). Serum levels of Tau-A and Tau-C were log-transformed to account for the skewness and then z-score standardized using the population mean and standard deviation (SD). In cause-specific Cox proportional hazards regression models, all-cause dementia and Alzheimer's disease were used as the dependent variables. Age was used as timescale and event-free mortality was included as a competing risk as outlined by Benichou and Gail[13]. Levels of the tau fragments were included as either a continuous variable to reflect the risk associated with a change of one SD on the log scale, or as a categorical variable either dichotomized at the median (the group below the median was used as reference) or divided into quartiles (the lowest quartile (Q1) was used as reference). Initially we modeled the crude risk in the separate univariate analysis (model 1). Secondly, in addition to age, we adjusted for education level (primary school, high school and university) (model 2). Lastly we made a multivariate model adjusted for the continuous variables; age (as timescale), body mass index (kg/m^2), platelet count (10^9/L), white blood cell count (10^9/L), albumin (mmol/L), alkaline phosphatase (unit/L), gamma glutamyltransferase (unit/L), high-density lipoprotein (mmol/L) and the categorical variables; education level, smoking (never, past or current), alcohol consumption (never, <10.5 alcohol units/week, 10.5-21 alcohol units/week or >21 alcohol units/week), physical activity (other than walking) (never, once weekly, twice weekly or three or more times per week) (model 3). Selection of covariates was based on significant association with levels of Tau-A and Tau-C using a multiple linear regression analysis (data not shown) and relevant risk factors as reported in the literature.

A sensitivity analysis was performed for Tau-A by including all samples between LLOQ and LLOD with an intra-assay CVs above the initial requirement of <15%.

The cumulative incidence of a dementia event in a competing risk framework taking the risk of death without dementia into account was illustrated in quartiles of Tau-A and Tau-C. The cumulative incidence was estimated using the Aalen–Johansen method[14]. The difference between cumulative incidence curves was tested using the modified χ^2 statistic outlined by Gray[15].

Finally we investigated the predictive value of the two biomarkers when added *i)* to a reference model containing age and educational level and *ii)* to a reference model containing all the independent variables from the multivariate model. The predictive value was assessed by computing the area under the Receiver-Operating Characteristics curve (AUC) for a 5-year and 10-year prediction horizon using time from baseline as timescale.

Results

Selected baseline characteristics of the study population are summarized in Table 1. During the follow-up period of maximum 15 years (median follow-up time 13.7 years) a total of 538 incident dementia cases were identified from the registries, of which 232 had AD.

(table 1 here)

Cause-specific Cox proportional hazards regression models were used to assess the association between the biomarker levels and the risk of incident dementia or AD as listed in Table 2. Higher levels of Tau-C, both as a continuous measure and categorized, were associated with a decreased risk of all-cause dementia and AD in the age-adjusted model. Subjects in the highest quartile had a 29% (HR [95% CI] 0.71 [0.55-0.91]) decreased risk of dementia and a 34% (HR [95% CI] 0.66 [0.46-0.96]) decreased risk of AD as compared to subjects within the lowest quartile. A dose-response tendency was observed for Tau-C in all three models, indicating decreasing risk of dementia with increasing levels of the biomarker.

(table 2 here)

The association between Tau-C and incident dementia and AD remained significant after adjustment for age and education and in the multi adjusted model. Tau-C levels in the highest quartile were associated with a 29% lower risk of dementia and 34% lower risk of AD, when adjusted for age and education. In the multi adjusted model, subjects in the highest quartile had a 24% (HR [95% CI] 0.76 [0.58-0.98]) lower risk of dementia as compared to subjects in the lowest

quartile. Further, the risk of dementia and AD decreased 10% (HR [95% CI] 0.90 [0.82-0.99]) and 13% (HR [95% CI] 0.87 [0.75-1.00]) with every log SD increase of the biomarker, respectively.
A dose-response relation was also observed with across the quartiles of Tau-A, and as continuous measure the risk decreased 13% (HR [95% CI] 0.87 [0.79-0.96]) in relation to all-cause dementia and 17% (HR [95% CI] 0.83 [0.72-0.96]) in relation to AD with every log SD increase of the biomarker in the age and educationally adjusted model, respectively. When dichotomized at the median subjects above the median had a 19% lower risk of dementia (HR [95% CI] 0.81 [0.67-0.98]) and a 32% lower risk of AD (HR [95% CI] 0.68 [0.50-0.91]). After multi factor adjustment the association between Tau-A and incident dementia and AD vanished, however the association remained significant between Tau-A and all-cause dementia as a continuous measure and in the dichotomized analysis, where subjects above the median had 29% decreased risk of AD (HR [95% CI] 0.71 [0.52-0.98]). The sensitivity analysis for Tau-A did not alter the overall results. Thus, there was a minor tendency for both outcomes where the HRs was shifted modestly towards the null (data not shown).

The two tau biomarkers were also stratified into quartiles and illustrated as cumulative incidence curves (Figure 1). The analysis showed that the separation between Q1 and Q4 for Tau-A in relation to all-cause dementia is poor, and with significant overlap between confidence intervals for Q1 and Q4 (Figure 1A, $p = 0.2$). For AD on the other hand, the separation between Q1 and Q4 is larger (Figure 1D, $p = 0.03$). For Tau-C, the separation between the quartiles is larger and with only minor overlap between the confidence limits of Q1 and Q4 (figure 1B, E, $p = 0.0009$ for dementia and $p = 0.01$ for AD). Moreover, a dose-response relation across the four quartiles is observed for Tau-C in relation to both dementia and AD. The overlay plots (figure 1C and 1F) illustrate Q1 and Q4 for both biomarkers. It appears that the distance and thereby the separation between Q1 and Q4 increases from Tau-A to Tau-C.

As outlined in table 3, Tau-A did not improve the risk prediction of dementia or AD within a 5-year and 10-year prediction horizon. Using a 5-year prediction horizon, Tau-C improved the prediction for both dementia and AD minimally, with an AUC change of 0.01 and 0.02, respectively ($p = 0.05$ for both), although only with model 2 as the reference model. In the 10-year prediction horizon Tau-C did not improve the prediction as compared to any of the reference models.

Discussion

In this study we assessed the prognostic utility of two novel serum biomarkers of neurodegeneration in a large prospective study. Both biomarkers, Tau-C in particular, were associated with incident dementia, where high levels of the biomarkers were associated with lower risk of incident dementia and AD.

The inverse association between levels of tau and risk of dementia seems counterintuitive since higher levels of tau are found in the CSF of subjects with AD and to a lesser degree in other types of dementia[16–18]. Sparks and colleagues, however, also found lower levels of tau in plasma of AD patients and explain their association with a reduced transport of excess central tau to the periphery, caused by pathological alterations of tau[19]. Likewise, a similar situation has been observed with the Glial Cell-Line Derived Neurotrophic Factor protein in AD subjects. Here, the protein level is decreased in serum and increased in CSF in AD versus control subjects. The authors speculate that it could be related to an altered function of the blood-brain barrier thus disturbing clearance or facilitating crossing of potentially harmful fragments in the healthy brain[20]. Another plausible explanation is linked to neuroinflammation where microglia exhibit significant phenotypic changes during the course of the disease. In early AD microglial activation is believed to be neuroprotective by enhancing phagocytosis and degradation of β-amyloid and tau[21,22], a process that may result in less release of tau to the periphery. In later stages, where microglia become over-activated, they lose their phagocytic abilities resulting in uncontrolled inflammation[23]. This would result in higher levels of both central and peripheral tau.

There are previous reports of measurements of tau protein in circulating blood, but most studies are small in size, low in numbers, and show inconsistent results[19,24–30]. A recent meta-analysis has therefore concluded that plasma tau is not a useful marker for AD[31]. The meta-analysis included six studies, whereof some reported an increase[25,27,28], others a decrease[19,24] and one study reported no change in AD patients as compared to healthy age-matched controls[26]. The heterogeneity across the studies illustrates one of the challenges of biomarker assessment in blood. An important difference between the previous studies and ours is that previous studies measured total tau and not truncated tau. Truncated tau appears to be specifically related to disease pathology and recent studies have shown the presence and elevation of several truncated tau species in CSF of AD patients[32]. Besides being more specific for pathological changes than the intact proteins, the truncated fragments might more easily pass through the blood-brain barrier, due to their smaller size, as larger fragments do not cross the barrier.

Another important difference is the setting in which the markers are assessed. With one exception[30], the previous studies of plasma tau are cross-sectional, while ours is longitudinal,

and thereby the first large cohort study to assess the prognostic utility of truncated tau in serum. Importantly the longitudinal design limit the concern of reverse causation. Mattson and colleagues recently touched upon the prognostic potential of plasma tau where they found that higher plasma tau was associated with progression, measured as the change in cognitive performance over time, however this was assessed in subjects with MCI and established dementia and not cognitively normal individuals [30]. The biomarker dynamics of Tau-A and Tau-C as a function of disease severity are still to be elucidated, but based on the current observations we speculate that the levels of the biomarkers are time-dependent and may change direction during the course of the disease. In minor cross-sectional studies of dementia and mild traumatic brain injury subjects, we found that the levels of Tau-A and Tau-C were elevated in diseased versus control subjects[8,9]. Associations with opposite direction as to what we found in this prognostic analysis. While the influence on the disease path after processing of tau by ADAM10 is unknown, evidence suggests that the caspase cleavage leading to the generation of Tau-C may play an important role in the cascade leading to tau aggregation[33]. The Tau-C fragment has been found to be one of the truncated tau forms in NFTs[7]. This evidence suggests that Tau-C may accumulate within the neurons during the process of NFT formation, and eventually be released to the circulation, at a more advanced disease stage, where the NFT load is sufficient to cause neuronal cell death. This process could explain the associations we have observed in our cross-sectional and longitudinal studies, respectively. There are previous indications of a non-linear relation between tau and disease severity over time. Using data from the ADNI database, Mouiha and colleagues investigated the time course of the CSF biomarkers, Aβ, t-tau and p-tau, and for all three markers the most likely model describing the relation between the biomarkers and disease severity was non-linear[34]. The most likely time course for t-tau and p-tau was found to be a penalized B-spline model, where multiple inflexion points could indicate multiple phases of accumulation as opposed to a continuous, uninterrupted process. Recent longitudinal data from the DIAN study also suggest that the biomarker trajectories may differ as a function of disease severity[35].

While the associations of Tau-C and Tau-A with incident dementia and AD revealed a potential value of these novel biomarkers, their predictive value as individual markers was limited. Tau-C gave a very modest increase in the AUC in a 5-year prediction horizon as compared to a reference model based on age and education, however the increase in AUC vanished in the fully adjusted model. It must be noted that this assessment was done in a population-based cohort without any specific enrichment e.g. a requirement for Aβ positivity. The heterogeneous population may leach out the predictive performance. Despite the limited predictive value as stand-alone biomarkers, it is likely that the markers could be useful in combination with other serum biomarkers e.g. other tau-species and β-amyloid. From our study it is clear that the interpretation of a peripheral signal and its relation to alterations within the brain is difficult, albeit studies, including the current study, begin to highlight that serological assessment of pathophysiological tau processing is possible.

Understanding the link between the biomarker signal and the pathophysiological processes is however of paramount importance and studies that can reveal the time-dependency and biomarker dynamics in relation to disease severity e.g. with repeated measures should therefore be a priority for the future.

A peripheral biomarker of biologically processed tau has several advantages as compared to β-amyloid. First, while the Amyloid Precursor protein is also expressed in peripheral tissues like the pancreas, kidney, heart and liver[36], animal studies suggest that circulating tau protein arises from central neurons[37]. This implies that the peripheral pool of tau would arise directly from the brain, while β-amyloid in plasma or serum probably reflects a mixture of peripheral and brain-derived protein. This might make the interpretation of a peripheral tau signal easier, although the processing, release and transport of tau from the brain to the periphery is yet not fully understood. Similar to a previous study of total tau measured in plasma, our markers did not show any correlation with t-tau or p-tau levels in CSF suggesting that the steady-state concentrations of tau are differentially regulated in these two body fluids[9,25]. Secondly, it has become quite clear that, although CSF Aβ aids in the early diagnosis of AD, the marker is not related to disease severity and duration[38,39]. CSF Tau, on the other hand, correlates with disease severity during the whole time course of AD[39–41]. An association that is also likely with truncated tau in the periphery. Finally, tau outperformed Aβ in a head-to-head comparison from a recent meta-analysis, where tau proved to have a larger effect size (measured by the disease to control ratio) in both CSF and plasma/serum[42].

Limitations

Generally the dementia field is hampered by misdiagnosis and underdiagnoses which complicate the evaluation of new diagnostic and prognostic biomarkers. In the current study we used registry-linkage to collect information on dementia diagnoses. This method has the advantage of a very limited loss to follow up, however one could question the validity of the diagnoses due to its origin. Similar registries are found in other countries in Scandinavia, and studies from Sweden and Finland have shown that the diagnoses in the registries have very good accuracy, but underestimation is present. This underestimation may result in an underestimation of the biomarker potential. [43,44]

Like most other studies we based our biomarker assessment on a binary distinction between cases and controls. Since dementia evolves over decades with a long preclinical phase the binary distinction is probably not the most appropriate method as the control group may contain several subjects with preclinical disease at the time of biomarker assessment. Although this is difficult to work around, a long follow-up time as in the current study, is one of the best possibilities to avoid misclassifications.

In literature, variability in assays and detection challenges are reported as two major hurdles with peripheral biomarkers that should be overcome before the full potential of these biomarkers is expressed[45,46]. At least for our Tau-A assay we also faced a challenge with sensitivity, which we hope to overcome with assay optimization. In a sensitivity analysis, we did not observed any significant impact on the overall findings of the samples measured below the LLOQ with intra-assay CVs above 15%. The current study was limited to women and therefore generalization cannot be made to men of same age. The biomarkers should be tested in other cohorts to ensure reproducibility and generalizability.

Conclusions

The current study demonstrates that serological assessment of pathophysiological tau processing is possible. Tau-A and Tau-C measured in serum could be useful prognostic biomarkers to aid in early diagnosis of preclinical dementia and AD. Additional validation in relation to prognosis and time-dependency of these novel biomarkers should be a subject for future investigations.

List of abbreviations

AD: Alzheimer's Disease

AUC: Area under the Receiver-Operating Characteristics curve

CI: Confidence Interval

CSF: Cerebrospinal fluid

CV: Intra-Assay Coefficients of Variability

HR: Hazard ratio

LLOD: Lower limit of detection

LLOQ: Lower limit of quantification

MCI: Mild Cognitive Impairment

NFT: Neurofibrillary tangles

PERF: the Prospective Epidemiological Risk Factor study

SD: Standard deviation

Declarations

Ethics approval and consent to participate: The protocol was approved by the research ethics committee of the Copenhagen County (approval reference: KA 99070gm). Written informed consent was obtained from all subjects prior to any study related procedures.

Consent for publication: Not applicable

Availability of data and materials: The datasets generated and analyzed during the current study are not publicly available as subjects did not consent to have their data publicly available. The datasets are available from the corresponding author on reasonable request.

Competing Interests: JSN, KDM and SB reports no disclosures. CC serves as board member and stock owner in Nordic Bioscience A/S. MAK and KH are full-time employees and hold stocks in Nordic Bioscience A/S. MAK and KH hold patent applications on the Tau biomarkers.

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Author contributions: JSN was the primary contributor in writing the manuscript including literature search, generation of figures, data and statistical analysis and the data interpretation. KDM was a contributor in writing the manuscript, data and statistical analysis and the data interpretation. CC was responsible for the study design and data collection and gave scientific advice. MAK contributed in writing the manuscript, data interpretation and gave scientific advice. SB was involved in writing and data interpretation. KH was involved in writing, data interpretation and gave scientific advice. All authors read and approved the final manuscript.

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Table 1: Study population characteristics at baseline. Numbers are shown as absolute numbers with percentile in brackets for categorical variables. For numerical variables the mean (standard deviation) is shown.

Parameter	Quartiles of Tau-A					Quartiles of Tau-C				
	Q1 n=1054	Q2 n= 1032	Q3 n=1034	Q4 n=1039	p value	Q1 n=1365	Q2 n=1314	Q3 n=1330	Q4 n=1300	P value
Tau-level, min-max (ng/mL)	9.3-20.5	20.6-26.9	27.0-35.9	36.0-199.3	NA	8.6-14.7	14.8-19.1	19.2-25.8	25.9-167.7	NA
Age, mean (SD) (years)	70.1(6.4)	70.1(6.6)	70.4(6.4)	71.2(6.5)	<0.001	70.6(6.3)	70.8(6.5)	70.7(6.5)	70.2(6.6)	0.2
Highest level of education										
Primary school, n (%)	730(69)	735(71)	723(70)	742(71)		938(69)	919(70)	947(71)	939(72)	
High School, n (%)	239(23)	222(22)	240(23)	220(22)	0.8	325(24)	290(22)	280(21)	273(21)	0.4
University, n (%)	84(8)	73(7)	69(7)	77(7)		98(7)	104(8)	101(8)	88(7)	
BMI, mean (SD) (kg/m^2)	25.0(3.9)	26.0(3.9)	26.9(4.2)	27.4(4.8)	<0.001	25.1(3.8)	25.8(3.9)	26.3(4.1)	27.5(4.8)	<0.001
Smoking										
Never, n (%)	509(48)	489(48)	493(48)	477(46)		708(52)	648(49)	610(46)	566(44)	
Past, n (%)	302(29)	319(31)	317(31)	309(30)	0.6	400(29)	406(31)	423(32)	379(29)	<0.001
Current, n (%)	242(23)	222(22)	223(22)	253(24)		255(19)	259(20)	296(22)	355(27)	
Alcohol										
Never, n (%)	414(40)	403(40)	444(43)	502(49)		530(39)	538(41)	541(41)	653(51)	
<10.5 units/week, n (%)	268(26)	257(25)	251(25)	227(22)	0.004	347(26)	325(25)	300(23)	280(22)	<0.001
10.5-21 units/week, n (%)	293(28)	285(28)	265(26)	236(23)		367(27)	351(27)	376(29)	290(22)	
>21 units/week, n (%)	70(7)	70(7)	66(6)	70(7)		109(8)	92(7)	103(8)	69(5)	
Physical activity										
Never, n (%)	297(28)	292(28)	314(30)	376(36)		366(27)	380(29)	411(31)	436(34)	
1 time/week, n (%)	213(20)	221(22)	222(22)	216(21)	0.001	273(20)	275(21)	298(22)	281(22)	0.004
2 times/week, n (%)	142(14)	141(14)	154(15)	118(11)		195(14)	177(14)	170(13)	160(12)	
3+ times/week, n (%)	401(38)	376(37)	343(33)	327(32)		529(39)	480(37)	450(34)	422(33)	
Hypertension, n (%)	278(27)	304(30)	332(32)	372(36)	<0.001	393(29)	394(30)	400(30)	430(33)	0.1
Hyperlipidaemia, n (%)	96(9)	80(8)	100(10)	92(9)	0.5	98(7)	134(10)	125(9)	106(8)	0.03
Diabetes, n (%)	17(2)	26(3)	33(3)	40(4)	0.06	30(2)	35(3)	30(2)	51(4)	0.04
Depression/Anxiety, n(%)	64(6)	67(7)	66(6)	78(8)	0.6	83(6)	89(7)	97(7)	76(6)	0.4

Table 2: Association of Tau-A and Tau-C with the risk of incident dementia and Alzheimer's disease.

			Continuous (per log SD decrease)			Dichotomized at median			Quartiles								
						> (Ref: <)			Q1	Q2			Q3			Q4		
Biomarker		Outcome	HR	95% CI	P	HR	95% CI	P	HR	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Model 1	Tau-A	Dementia	0.87	0.79-0.96	**0.005**	0.81	0.67-0.98	**0.03**	Ref	0.94	0.72-1.22	0.6	0.83	0.63-1.09	0.2	0.74	0.57-0.98	**0.03**
		AD	0.83	0.72-0.96	**0.01**	0.68	0.50-0.91	**0.01**	Ref	0.82	0.56-1.20	0.3	0.63	0.41-0.95	**0.03**	0.61	0.40-0.91	**0.02**
	Tau-C	Dementia	0.88	0.80-0.96	**0.005**	0.79	0.67-0.94	**0.007**	Ref	0.89	0.71-1.11	0.3	0.80	0.64-1.01	0.06	0.71	0.55-0.91	**0.006**
		AD	0.83	0.72-0.95	**0.009**	0.71	0.55-0.93	**0.01**	Ref	0.84	0.60-1.18	0.3	0.66	0.46-0.94	**0.02**	0.66	0.46-0.96	**0.03**
Model 2	Tau-A	Dementia	0.87	0.79-0.96	**0.004**	0.81	0.67-0.98	**0.03**	Ref	0.94	0.72-1.22	0.6	0.83	0.63-1.09	0.2	0.74	0.57-0.98	**0.03**
		AD	0.83	0.72-0.96	**0.01**	0.68	0.50-0.91	**0.01**	Ref	0.82	0.56-1.21	0.3	0.63	0.41-0.95	**0.03**	0.61	0.40-0.91	**0.02**
	Tau-C	Dementia	0.88	0.80-0.96	**0.006**	0.79	0.67-0.94	**0.008**	Ref	0.89	0.71-1.12	0.3	0.81	0.64-1.02	0.07	0.71	0.56-0.91	**0.008**
		AD	0.83	0.72-0.95	**0.008**	0.71	0.55-0.93	**0.01**	Ref	0.84	0.60-1.17	0.3	0.66	0.46-0.94	**0.02**	0.66	0.46-0.96	**0.03**
Model 3	Tau-A	Dementia	0.89	0.81-0.99	**0.03**	0.85	0.70-1.04	0.1	Ref	0.98	0.75-1.28	0.9	0.88	0.66-1.16	0.4	0.79	0.59-1.06	0.1
		AD	0.87	0.74-1.01	0.07	0.71	0.52-0.98	**0.03**	Ref	0.89	0.60-1.32	0.6	0.67	0.44-1.04	0.07	0.67	0.43-1.04	0.07
	Tau-C	Dementia	0.90	0.82-0.99	**0.03**	0.84	0.70-1.00	**0.05**	Ref	0.89	0.71-1.12	0.3	0.84	0.66-1.06	0.1	0.76	0.58-0.98	**0.03**
		AD	0.87	0.75-1.00	**0.05**	0.78	0.60-1.03	0.08	Ref	0.87	0.62-1.23	0.4	0.72	0.50-1.05	0.09	0.75	0.51-1.11	0.1

Ref: reference group. Model 1 was adjusted for age. Model 2 for age and education. Model 3 was adjusted for: age, education level, body mass index, smoking (never, past or current), alcohol consumption (never, <10.5 alcohol units/week, 10.5-21 alcohol units/week or >21 alcohol units/week), physical activity (other than walking) (never, once weekly, twice weekly or three or more times per week) platelet count, white blood cell count, albumin (mmol/L), alkaline phosphatase (unit/L), gamma glutamyltransferase (unit/L), high-density lipoprotein (mmol/L). P≤0.05 are marked with bold.

Figure title and legend

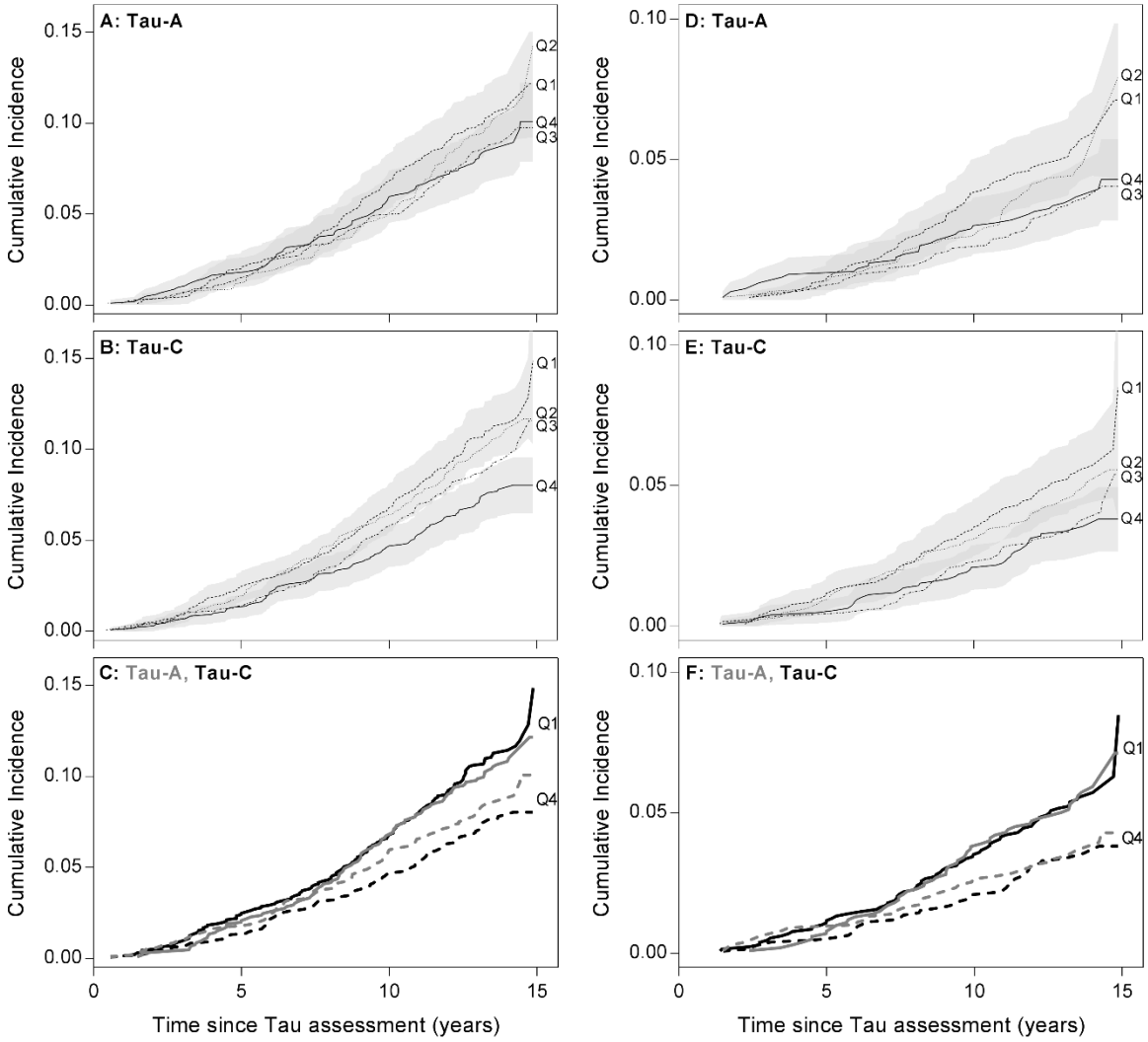

Figure 1: Cumulative incidence curves. Cumulative incidence in quartiles of Tau-A and Tau-C as a function of follow-up time. Left panel (A-C) illustrates the cumulative incidence for all-cause dementia. Right panel (D-F) illustrates the cumulative incidence for AD. The bottom graphs (C and F) are overlay plots of Tau-A and Tau-C showing only Q1 and Q4 for both markers. Confidence intervals (95%) are shown for the lowest (Q1) and highest quartiles (Q4) in A, B, D and E.

8

Summary of Results

8. Summary of Results

The presented studies found that age, lifestyle factors such as BMI, level of physical activity, fasting plasma glucose levels and total cholesterol levels as well as history of depression and cognitive test performance are important predictors of dementia in elderly women from the PERF cohort. The results are summarized in table 6.

Table 6: Overview of significant findings from paper II-V. The papers reporting the findings are listed. OCI: Objective Cognitive Impairment, BMI: Body Mass Index, HR: Hazard ratio, HOMA-IR: Homeostasis Model Assessment Index, OR: Odds ratio, CI: Confidence Interval

Predictor	Description of association	Reported in
Age	Age was a strong risk factor for all-cause dementia and for differential diagnoses (HR 2.05 [1.89-2.21])	Paper II
Cognitive Performance	Subjects with Objective Memory Impairment (OCI) had elevated risk of dementia (HR 1.46 [1.19-1.79]).	Paper IV
Body Mass Index	Overweight (BMI 25-30 kg/m^2) was associated with lower risk of dementia (0.75 [0.62-0.89]), AD and OD.	Paper II
Smoking Habits	Smoking increased the risk of VaD (HR 2.56 [1.18-5.55])	Paper II
Physical Activity	Physical activity reduced the risk of dementia (HR 0.77 [0.61-0.96]) and was associated with lower risk of progression from OCI to dementia/severe impairment (OR 0.29 [0.09-0.94])	Paper II Paper IV
Alcohol Consumption	Lower risk of progression for light alcohol consumers as compared to abstainers (Abstainers OR 1.60 [1.06-2.42])	Paper IV
Fasting Plasma Glucose	Dose–response relationship between FPG and risk dementia. Impaired FPG had larger odds of cognitive dysfunction (OR 1.42 [1.09-1.84])	Paper II Paper III
Insulin Resistance	Subjects with insulin resistance have higher probability of developing cognitive dysfunction (OR 1.55 [1.16-2.07])	Paper III
Number of MetS risk factors	Presence of several metabolic risk factors elevated odds of cognitive dysfunction (OR 2.98 [1.10-8.07])	Paper III
Total Cholesterol	Higher total cholesterol associated with risk of progression to dementia/severe impairment (OR 4.82 [2.01-11.5])	Paper IV
History of Depression	Depression associated with dementia (HR 1.75 [1.32-2.34]) and risk of progression to dementia/severe impairment (OR 4.72 [1.02-21.8])	Paper II Paper IV
Tau-A and Tau-C	High levels of Tau-A and Tau-C associated with lower risk of dementia (Tau-A: HR 0.85 [0.70-1.04]; Tau-C: 0.84 [0.70-1.00]).	Paper V

Paper II assessed risk factors for all-cause dementia and differential dementia diagnoses in a study population free from dementia at baseline, while **Paper III** specifically studied metabolic risk factors and their relation to cognitive dysfunction in a sub-cohort free from dementia and cognitive dysfunction at baseline who also attended the follow-up visit. These studies found that BMI in the overweight range and physical activity were associated with lower risk of dementia (Paper II), while age, history of depression, insulin resistance and elevated fasting plasma glucose increased the risk of incident dementia (Paper II or Paper III). **Paper IV** included subjects at different stages on the cognitive continuum. The degree of OCI was found to be associated with risk of dementia

in a study population free from dementia and severe cognitive impairment at baseline. In subjects with OCI at baseline, **Paper IV** also revealed that physical inactivity, elevated total cholesterol levels and a history of depression were all associated with progression to dementia or severe cognitive impairment, while abstinence from alcohol consumption was associated with progression in a larger study population including also those with normal cognitive test performance at baseline. **Paper V** introduced two novel biomarkers measuring truncated tau protein in the circulation. The study showed that Tau-A and Tau-C were associated with risk of dementia and thereby potentially important predictors of dementia. As individual markers, the predictive performance was very modest although Tau-C did result in a small improvement in the risk prediction compared to a reference model with age and level of education. The markers may have potential for identification of high-risk subjects if used in combination with other peripheral biomarkers and risk factors. Overall, the presented papers contribute to extend knowledge on dementia and cognitive impairment specifically in elderly Danish women.

The nomogram in figure 10 illustrates a simple tool useful for individually tailored risk prediction as it is based on the risk profile of an individual subject. In the context of this thesis it is thought as an example of how assessment of risk factors can be translated into a simple and useful tool for population screening or in the everyday clinic. The nomogram is used to manually obtain prediction by making vertical readings from each of the variables to the point scale on the upper part of the nomogram. Finally, all readings from individual variables are summarized and the predicted risk is read vertically from the total points scale to the predicted risk scale.

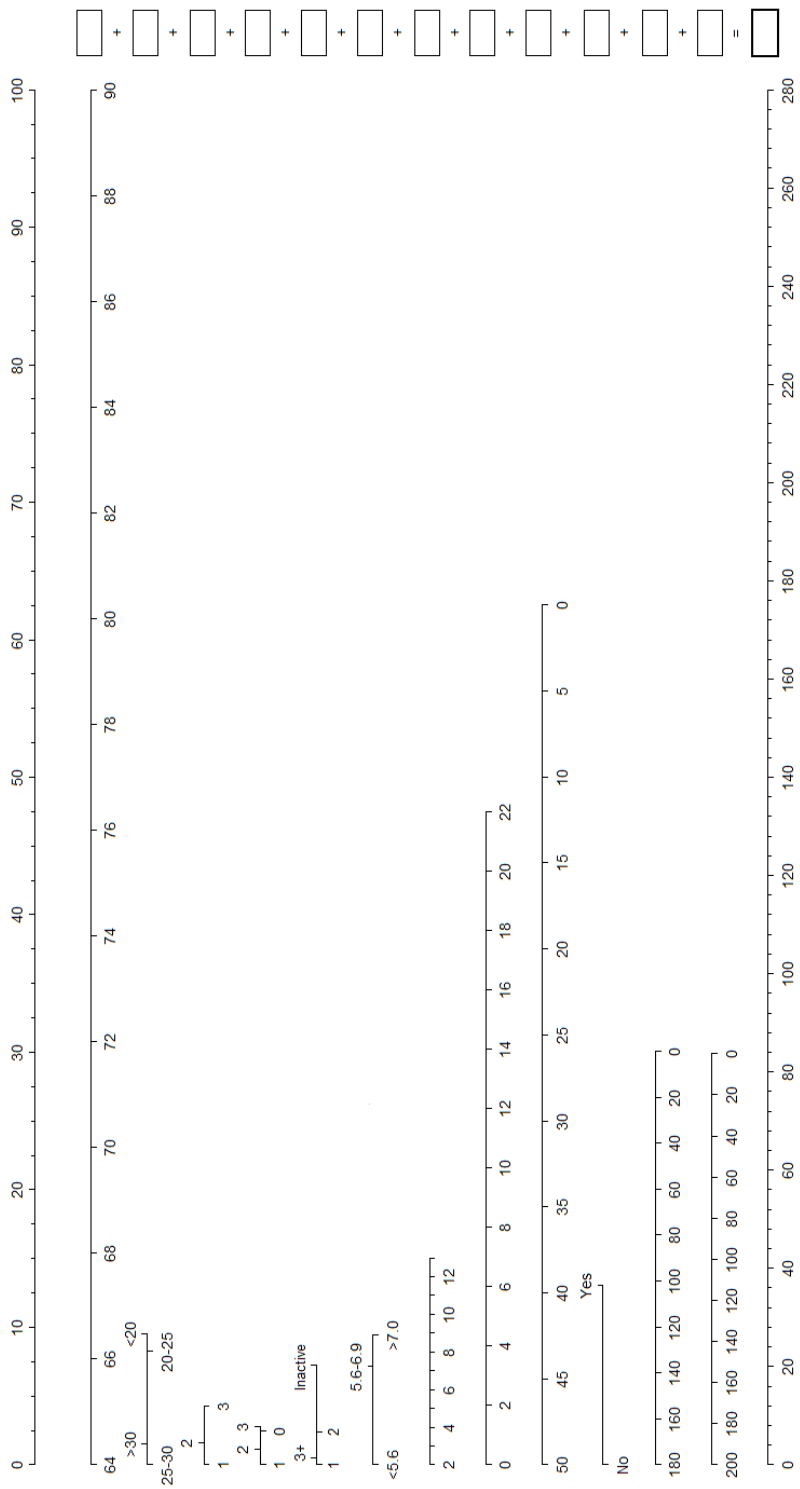

Figure 10: Nomogram, a tool for individual risk prediction. The contribution from each risk factor is read on the "Points" scale on the top of the figure using vertical lines. All individual points are summarized and the total score is used to assess the risk of dementia in either a 5-year, 10-year or 15-year prediction horizon again using a vertical line.

The nomogram was generated from a multivariate Cox proportional hazards regression model including all of the independent predictors depicted in the nomogram. The variables were selected based on findings from paper II-V. Insulin Resistance was excluded from the analysis as blood levels of insulin was only available for the sub-cohort attending the follow-up study and as the purpose of the screening tool was to identify all-cause dementia, information on smoking was not included. At present, no effort was made to simplify the underlying prediction model and it has not been validated in any independent cohorts. The accuracy was assessed by computing the area under the Receiver-Operating Characteristics curve (AUC) for a 5-year, 10-year and 15-year prediction horizon. The AUCs were: 0.79 (5-year), 0.76 (10-year) and 0.66 (15-year). What is clear from the nomogram is that age is the single most important predictor of dementia followed by cognitive test performance and level of the tau biomarkers. The other risk factors individually add little to the overall predicted risk. As an example, the nomogram was used to predict risk for a 75-year old women with either a low-, intermediate- or high-risk profile for development of dementia in late-life (table 7). The number of points was read manually by making vertical lines on the nomogram for each individual predictor.

Table 7: Predicted risk for a 75-year old women with a low-, intermediate- or high-risk profile.

Risk Factor	**Low Risk**		**Intermediate Risk**		**High Risk**	
	Profile	Points	Profile	Points	Profile	Points
Age (years)	75	42	75	42	75	42
Body Mass Index (kg/m^2)	28	0	24	8	18	10
Level of Education	2	2	2	2	2	2
Alcohol Consumption (units/week)	8	0	13	2	25	3
Physical Activity (times/week)	3	0	2	2	0	7
Fasting Plasma Glucose (mmol/L)	5.0	0	6.1	8	7.5	10
Total Cholesterol (mmol/L)	5.0	5	7.0	7	10.0	11
Short Blessed Test (Score)	4	8	8	18	18	39
Category Fluency Test (Score)	40	12	25	31	12	48
History of Depression (yes/no)	No	0	No	0	Yes	13
Level of Tau-C (ng/mL)	80	16	40	24	20	27
Level of Tau-A (ng/mL)	100	15	100	15	20	27
Total Score		**98**		**159**		**239**
Prediction Horizon						
5-year		≈ 0		≈ 0		≈ 40
10-year		≈ 0		≈ 10		≈ 85
15-year		≈ 0		≈ 20		> 90

9

General Discussion

9. General Discussion

The manuscripts included in this thesis have investigated a range of risk factors and possible predictors of dementia in late-life, including two novel serum biomarkers. With specific focus on elderly women, the findings have added to the previous evidence base and additionally presented potential new tools for the identification of at-risk individuals. The thesis supports the idea that dementia disorders, AD in particular, are complex multifactorial conditions and that the etiology should be viewed in a life-course perspective. The specific findings from each manuscript are discussed within the individual manuscripts. In this general discussion, the findings are put in context to prevention of dementia. Also, some project related and general methodological challenges that hamper the transition from observation to action are discussed.

To set the scene we will start by outlining the estimated impact of successful prevention. A report from Alzheimer's Research UK estimates the impact of a 5-year delay in dementia onset. In absolute numbers this would mean 666,000 fewer people with dementia by 2050 in the United Kingdom alone, a reduction by 33% [187]. In cost savings, this would translate to £21.2 billion. Further it has been estimated that around one-third of AD cases worldwide can be attributed to seven modifiable risk factors; low educational attainment, physical inactivity, smoking, midlife hypertension, midlife obesity, diabetes, and depression [188]. An intervention on each of these risk factors resulting in a prevalence reduction by 10% would potentially reduce the worldwide AD prevalence by 8% (8.8 million cases) in 2050. This illustrates that even a slight delay will have great social and economic impact.

9.1 Risk and protective factors in relation to prevention

Age is the main risk factor for dementia [27], and the huge impact of this non-modifiable risk factor previously made some researchers speculate that an intervention on one or several of the modifiable risk factors will not be effective as a prevention strategy [189]. The relative importance between the risk factors in this thesis, as illustrated in the nomogram, supports age as the most important risk factor for dementia. Many of the modifiable risk factors have minimal individual contribution to the overall risk prediction, however as these modifiable risk factors often are co-expressed and tend to accumulate over time, they are still important in relation to prevention. Likewise, previous evidence suggests that clustering of certain risk factors implies synergistic effects [190]. The extensive research on modifiable risk and protective factors suggest that there might be a critical time period where the risk factor exerts its greatest impact on the future risk of dementia. The possibility that risk factors may change over time challenges the ability to draw firm conclusions for many of the risk factors reported in the literature, and is likely one of the reasons why the current evidence has not yet been translated into proper prevention strategies

[191]. Another important aspect is that risk factors may interact, which further challenges the possibility of establishing true cause effect relationships and therefore also the ability to identify the most important risk factors and appropriate time point for prevention. Albeit these challenges the nonpharmacological prevention strategies may very well represent the type of intervention with the largest potential effect in relation to dementia.

With the introduction of the life-course approach to the etiology of late-onset dementias [192], it has become even more obvious that risk factors build up across the lifespan and accumulate from midlife, where also the underlying pathological alterations are starting to accumulate in the brain [154]. Therefore, many speculate that this is the “window of opportunity” for prevention [193]. The primary risk factors in midlife are the lifestyle-related cardiovascular factors like hypertension, obesity, type 2 diabetes, physical inactivity and smoking, which are also the risk factors with the highest and most consistent level of evidence [62]. These factors are therefore speculated to be the factors with the largest potential effect on dementia prevention. Historically the attempts to translate and confirm the findings from the observational studies in RCTs have been disappointing. With few exceptions, single intervention RCTs have consistently failed to identify efficacious pharmacological (e.g. lipid-lowering, antihypertensive, hormone replacement therapy, non-steroid anti-inflammatory drugs) or nonpharmacological interventions [194,195]. Physical activity is indeed a single intervention where researchers have been able to translate the findings from observational studies to RCTs with success. This has been shown across the cognitive continuum, e.g. in subjects with MCI [196], and recently in the ADEX trial where supervised moderate-to-high intensity exercise was found to reduce neuropsychiatric symptoms and potentially preserve cognition in patients with mild AD [197]. The positive effect could be related to the multidomain effects of physical activity on both neurogenesis, vascular risk factors, inflammation and depressive symptoms [198]. There are other examples of successful single intervention RCTs involving cognitive training and nutrition, however, because of the heterogeneity between studies, including different outcome measures, type of intervention, timing of intervention, duration of intervention and length of follow-up, cross-study comparisons are difficult. These methodological challenges are also speculated to be one of the reasons for the lacking preventive recommendations [199].

The disappointing results from single intervention studies, the evolving recognition of dementia as a multifactorial syndrome and the life-course approach therefore initiated a transition from single intervention into a new era with multidomain intervention in the search for successful prevention strategies [193]. Three recent studies have tested non-pharmacological multidomain interventions that simultaneously target several modifiable risk factors. Two studies, the FINGER and the MAPT studies, reported positive outcomes showing modest improvements in cognition in the intervention groups [75,76]. In the FINGER study the multidomain invention was: diet, exercise, cognitive training, vascular risk monitoring while the intervention in MAPT consisted of

nutritional counselling, physical exercise and cognitive stimulation, in combination with omega-3 fatty acid supplementation. The reported findings are very promising, however whether these interventions will delay cognitive decline and dementia in the long term is still unknown. The preliminary results from the third study, the PreDIVA study, testing a nurse-led 6-year intervention with multidomain cardiovascular tailored lifestyle advice, discouragingly showed no overall effect on the incidence of all-cause dementia [200]. We therefore still remain to see an effect on dementia incidence, an effect that would provide robust proof of concept for these multidomain interventions as preventive strategies. Interestingly, both the FINGER and the MAPT studies have on-going follow-up studies with the primary aim to assess the long-term efficacy on dementia and AD incidence. Each of these trials differed in a methodological sense as they used different outcome measures, different recruitment strategies, different duration of intervention and varying length of follow-up. Therefore, these studies should be seen as the very start of this new era where improvement and standardization of trial methods are of paramount importance for successful prevention.

Primary prevention appears to be feasible as a stabilization or even a potential decline in dementia prevalence has been observed in both Western Europe and the United States [201,202]. Although the causes behind the reductions have not been confirmed, researchers speculate that the decline has been driven by an increase in educational attainment and a positive repercussion from the large efforts on cardiovascular disease prevention in the western world [203]. Opposed to the positive trends in dementia and cardiovascular disease prevalence the prevalence of obesity and diabetes is still increasing, and as diabetes is considered a major risk factor for dementia, prevention of diabetes could potentially reduce dementia prevalence even more [204]. Despite the declining trend in high-income countries, the worldwide burden of dementia will still pose a major threat to society especially in low-to-middle income countries where unhealthy lifestyles and less focus on non-communicable disease prevention has led to an increase in dementia prevalence [6,205].

The RCTs represent the highest level of evidence and are therefore often considered a necessity on the path towards dementia prevention. This is primarily supported by the fact that RCTs generally are the best way to determine causality [206]. In the context of dementia prevention the RCTs have limitations given the substantial duration of an intervention and the long timescales needed to attain a meaningful clinical endpoint [207]. Due to the lack of sufficiently qualified biomarkers that may act as surrogate endpoints the RCTs may therefore not always be the optimal choice [199]. Also ethical issues may limit the applicability of RCTs as known risk factors cannot be left untreated in the control group. The alternative, the observational studies, also have limitations in relation to potential bias, confounding and causation. Although, the longitudinal design generally limits the concern of reverse causation, it cannot be ruled out because of the

long latent phase of dementia in particular if the studies are not properly designed. The MELODEM guidelines highlight a range of common methodological challenges that arise specifically in studies of cognitive decline and dementia [208], challenges that may compromise the translation of research findings into preventive strategies. The guideline outlines methods for reporting and evaluating potential sources of bias and is therefore a promising framework towards high quality standardized studies.

9.2 Identifying at-risk individuals: Moving to the individual level

Statistical associations and trends are important in medicine and public health, but do not necessarily translate into good individual level associations. In other words there is no guarantee that associations found at the group level will be applicable for every individual. Moving to the individual level is therefore important for proper risk prediction in the general population. Risk prediction at the individual level is applicable for two main reasons: *i)* targeting of preventive measures to high-risk individuals [209] and *ii)* recruitment and enrichment of high-risk subjects in clinical trials [210]. A recent paper by Hampel et al. [93] stated that this can be achieved through the assessment of modifiable and non-modifiable risk factors, cognitive profile, biomarker proof of disease and changes of these factors over time. During the past decade, a range of risk scores for the prediction of dementia has been developed. The development was spearheaded by researchers from the Finnish Cardiovascular Risk Factors, Aging, and Dementia (CAIDE) study who published the CAIDE Dementia Risk Score in 2006 [209]. The score was specifically developed to predict dementia among middle aged people using a 20-year prediction horizon and has the strength of being externally validated [211]. Other risk scores target older populations using shorter prediction horizons [210,212]. The methodological approach is very similar across the different studies where a prediction model is built by evaluating a range of candidate predictors using primarily Cox proportional hazard regression models. The relative size of the parameter estimates is then used to convert the estimates into an easily interpretable scoring system. The absolute differences in accuracy between the various prediction tools are relatively small, although prediction tools developed for use in late-life tend to have slightly higher accuracy underlining the understanding that an event, in this case dementia, is easier to predict closer to time of occurrence [213].

Our nomogram has a number of similarities with the previous prediction tools. Age is consistently included in all prediction tools as the most important predictor [209,210,212,214]. We found that cognitive test performance was the second most important predictor, which is consistent with the Late-Life Dementia Risk Index and the Brief Dementia Risk Index [210,214]. As compared to the other late-life prediction tools our study is characterized by a longer follow-up period minimizing the risk of misclassification bias. Rounding to the nearest integer was used to obtain risk scores for each of the predictors in the previous prediction tools, while we used the exact parameter

estimates in the generation of the nomogram ensuring the most accurate reflection of the risk. Several of the previous prediction tools may also suffer from survival bias as the outcome assessment was based on attendance to one or several re-examinations during the follow-up period [209,212]. Survival bias is less likely in our study as we used registry-linkage to obtain information on the dementia diagnosis. This method of follow-up is less sensitive to attrition and is independent of the outcome and the risk factor profile at baseline.

Interestingly, we included two serum biomarkers of tau that in our analysis proved to be associated with dementia, a novel approach not previously seen with dementia prediction tools. Thereby our nomogram combines knowledge on modifiable risk factors, cognitive performance and neurodegenerative biomarker assessment, a combination that is believed the first critical step towards implementing precision medicine in the dementia field [93]. A question that remains is whether a single prediction tool can be applied across the lifespan, the cognitive continuum and in different settings. This is likely not possible as it is well-known that the modifiable risk factors may have important interactions with time and genes that are difficult to incorporate under the assumptions underlining the available prediction models. Also, as we observed in our studies it may not necessarily be the same prognostic markers in subjects with MCI as compared to a preclinical or healthy population. Finally varying accuracies have been observed across different settings e.g. in memory clinics where the prevalence is high, some tools perform well while the performance in the general population can be less accurate [215].

The current trend in pharmacological prevention trials is that study populations are enriched by means of biomarker positivity. The A4 study (NCT02008357) is enrolling asymptomatic or very mildly symptomatic individuals who have biomarker evidence of Aβ deposition while the TOMMORROW trial (NCT01931566) is including asymptomatic high risk subjects carrying the *APOE* and *TOMM40* risk genes [73]. The individual risk of each subject is assessed using an algorithm based on their age, cognitive performance at enrollment and the above mentioned genotypes. This may be considered precision medicine, however it can also threat the external validity of these trials since an excessive enrichment e.g. on less prevalent genotypes, potentially can limit the generalizability to the overall population. Importantly, the use of Aβ deposition for enrichment may also result in a significant number of misdiagnoses, as about one third of very old people without dementia or cognitive impairment show signs of Aβ pathology [216].

Another unexplored potential use of multivariate prediction tools is risk-stratified analyses in RCTs. The risk-stratified analysis is, in contrast to the conventional subgroup analysis, taking several patient attributes into account simultaneously, which does not only increase the power of detecting a treatment effect but also facilitates precision medicine [217]. This strategy is currently

being tested in the above mentioned TOMMORROW trial where the risk genes are used to assign trial subjects into low- and high-risk groups [74].

9.3 Assessment of tau and the utility of blood-based biomarkers

The ultimate aim with precision medicine is to develop tailor made interventions for individual patients. Blood-based biomarkers are potential cost-effective tools that may aid in precision medicine by: *i)* identification of patients who are in greatest need and who may benefit the most from a given treatment, *ii)* identification of patients who respond optimally to a specific treatment (safe and efficacious treatment), *iii)* identification of the optimal treatment for selected subpopulation of patients, and *iv)* efficient use of health care resources [218]. The most obvious use of blood-based biomarkers is for screening in medical practice, a setting where the large-scale use of CSF and imaging biomarkers is limited.

The development of blood-based biomarkers for dementia is still lagging behind the CSF biomarkers. The perceived main reason is the natural barrier, limiting for the transfer of substances from the brain to the blood. In contrast, the CSF can communicate freely with the brain interstitial fluid where neurons are known to secrete proteins such as tau [219]. Importantly, there is a certain degree of protein exchange between the brain and the periphery. First, there is the active and passive transport over the BBB, which also functions as a clearance system that removes waste materials from the brain [220]. Both AD and vascular pathology have been associated with increased BBB permeability, that potentially may enable the passage of small and lipid soluble molecules that would otherwise not be able to pass [221,222]. The ability of tau and truncated tau species to cross the BBB has also been shown very recently, emphasizing that blood levels of tau can indeed be useful as a biomarker [223]. Secondly, other studies have revealed a novel path for the clearance of macromolecules from the brain. The studies confirmed the presence meningeal lymphatic vessels that together with a glymphatic clearance pathway facilitates the drainage of CSF to the periphery [165,224,225]. This glymphatic system has been shown to be an important route of clearance for both Aβ and tau [164,224]. If these pathological agents are transported between the glymphatic and lymphatic systems are still unknown. These findings support further research in developing brain-derived proteins as biomarkers for dementia-related diseases.

In general, the previous studies of peripheral tau have been challenged by the low protein concentration in the circulation. Total tau is found at very low concentrations in the CSF approximately around 300-500 pg/ml and the plasma levels are approximately 100 times lower [226,227]. Due to the very low abundance, the assessment in plasma requires ultrasensitive technologies and highly specific antibodies to detect the protein. Albeit concerns when making cross study comparisons where findings are based on serum versus plasma we have shown that

the Tau-A and Tau-C fragments are measurable by standard enzyme-linked immunosorbent assay techniques [169,228]. This underscores the fact that smaller protein fragments, due to their molecular size, may more easily be transported from the brain to the periphery, as compared to their intact counterparts. A lack of correlation between the tau levels in the CSF and blood has been demonstrated [170,227] and also the half-life of tau is found to be much shorter in blood compared to the CSF [229,230]. This suggests that the protein is cleared through different mechanisms in the two compartments and implies that the understanding of the mechanism of release, the potential subsequent processing, and the clearance of brain-derived proteins from the periphery are of key importance. The biomarker trajectories of Tau-A and Tau-C are presently unknown but jointly our data and previous data suggest that the association may be time and/or disease stage dependent. Knowing the trajectories are important in the validation of these biomarkers and could be investigated e.g. in a cross-sectional cohort including subjects across the cognitive continuum from normal aging to severe dementia. Another possibility would be to assess the trajectories in a longitudinal cohort with several repeated measures. As the biomarker levels are measured following a complex process including processing in the brain, release, subsequent peripheral processing and clearance, one could also speculate the use of a single-threshold rule does not reveal the full potential of these markers. Rather, the longitudinal changes as a function of progression or intervention should be tested. This has been found with the biomarker CA125 used within cancer, where longitudinal changes in contrast to using a single-threshold increase the accuracy in ovarian cancer screening and also lead to earlier identification [231,232].

The tau biomarkers may be useful for differential diagnosis especially since proteolytic cleavage is a hallmark of all dementias. By targeting the PTMs i.e. disease specific combinations of proteins and proteases, it can potentially allow for a better separation between the various dementia diagnoses. CSF p-tau is a well-described example, as it has been found to be almost exclusively elevated in AD as compared to a range of other neurodegenerative diseases including other tauopathies [219]. Abnormal excessive phosphorylation of tau is found in many neurodegenerative diseases, and since as many as 85 phosphorylation sites have been identified in the tau molecule, it may be that the specificity for AD is related to some sort of disease-specific phosphorylation [233]. In line with this, it was previously shown that our Tau-A biomarker was able to separate AD patients from other types of dementias [170], suggesting that the combination of a specific protein and a specific protease provides additional information that reduces the crossover between the different pathologies.

The paradigm shift in AD diagnosis from a clinical-pathological to a clinical-biological entity has mainly been possible due to advances in the biomarker field. The core CSF biomarkers: $A\beta_{1-42}$, t-tau and p-tau as well as MRI and PET-amyloid imaging are all qualified by the European

Medicines Agency for enrichment in regulatory trials [234]. Although the regulatory authorities are beginning to recognize the use of biomarkers in clinical trials of AD, none of the biomarkers are yet qualified as diagnostic tools, outcome measures or longitudinal measures as the current data is not sufficiently strong to support this [234]. To obtain such qualifications, there is an on-going validation and standardization process, where global standards for both pre-analytical and analytical aspects are being developed to ensure reproducibility and consistency within laboratories, across laboratories and across different kit manufacturers [235–237]. The measurement of CSF biomarkers is associated with high costs and requires an invasive procedure. These limitations justify the development of blood-based biomarkers as these are considered more relevant for large-scale use [71]. If properly validated, blood-based biomarkers could be implemented as a first step in a multi-stage screening process for clinical trials as outlined by Henriksen et al. [166]. Such test would apply a stringent filter in the enrolment process by ruling out subjects without disease and thereby decrease the number of screen-failures that would otherwise have undergone more invasive and costly procedures like neuroimaging or CSF sampling. Current evidence suggests that blood-based biomarker panels are able to identify progression from MCI to AD and also from cognitively normal to some level of cognitive impairment [238,239]. Similar to the CSF biomarkers there is also an on-going standardization process for blood-based biomarkers [240]. There has indeed been a lot of progress with blood-based biomarkers recently and if the progress continues, it could mean that the blood-based biomarkers may very well have the potential to bypass the CSF biomarkers.

9.4 Strengths and Limitations

9.4.1 Bias and Confounding

A significant proportion of people with dementia do not receive a clinical diagnosis at any time or do so only late in the disorder where it is often too late to intervene. Primary care is considered the gateway to early diagnosis, however the diagnostic sensitivity in primary care is estimated to be as low as 0.09 for subjects presenting with few or mild symptoms of dementia [241]. Under-diagnosis has also been found to increase with age [242], which can be worrisome as dementia incidence increases exponentially with age. Results from the Canadian Study of Health and Aging previously estimated 64% of undetected dementia in community-dwelling older people [243]. This indicates that dementia clearly is underdiagnosed and this underestimation will cause misclassification bias in epidemiological studies, like the PERF study, that do not have a comprehensive diagnostic workup. Even delayed diagnosis may lead to misclassification bias, however this can be minimized with a long follow-up period as in PERF. The causes of under-diagnosis in primary care are many and include; limited time with the patient, lack of standardized validated screening protocols, and unfeasible assessment tools in practice [241]. This indicates that short cognitive screening tools like those used in the PERF study are relevant tools in primary

care. Whether these tools are the most appropriate from a research perspective can of course be questioned, but in the transition from observation to action, they are highly relevant.

In paper III and IV we used information obtained at the follow-up visit (PERF II) to determine the outcome. This can lead to survival bias which is not uncommon in longitudinal studies of dementia. In contrast we used registry-linkage in paper II and V, a method characterized by very limited loss to follow-up and thereby less inflicted by survival bias. In the analyses using registry-linkage we were also able to take into account the competing risk of dementia-free mortality which can be a problem in longitudinal studies of such advanced age, especially when it comes to prediction [244]. A previous study specifically assessed the validity of dementia diagnose in the Danish national registries [245]. They found a validity of 86% for all-cause dementia and therefore concluded that registry-linkage is suitable for use in epidemiological dementia studies.

The two outcomes used are not interchangeable and this should be kept in mind as we included findings based on both outcomes in our nomogram. The use of cognitive tests to define the outcome is associated with large variation and the performance can be affected by many factors, which are not necessarily due to the disease of interest [246]. Due to the long latent phase of dementia preceding the clinical diagnosis, some of the observed associations may be caused by reverse causation, a problem particular relevant to consider in late-life studies were the exposure may be assessed close to the outcome. Given the previously reported interconnection between genetic and modifiable risk factors, the lack of genetic risk factors in our studies is a limitation that could result in unmeasured confounding. For example it has been reported that carriers of the *APOE* ε4 allele are more vulnerable to certain risk factors, while some protective factors like high education and physical activity can counteract the genetic risk [247].

Our study on cognitive trajectories from paper IV is limited by only two time points and a relatively simple cognitive assessment. Repeated cognitive assessments e.g. with one or two year intervals during the follow up period would have been optimal as it would have given us the opportunity to study the rate of progression in more detail. The evaluation of the tau biomarkers would also have benefited from such a study design especially if repeated cognitive assessments were accompanied by repeated biomarker measurements.

9.4.2 Reliability of the clinical diagnosis

Several studies have investigated the concordance between clinical dementia diagnoses and neuropathological changes at autopsy [20,21,248]. As an autopsy confirmed diagnosis remain s the gold-standard in the field, these studies are particular important as both epidemiological and biomarker studies mostly rely on the clinical diagnosis. The sensitivity and specificity varies between the studies but some are as low as 50%, indicating that the clinical diagnosis does not

always correspond to the diagnosis at autopsy. Importantly, it gives an indication of what accuracy we can expect when evaluating new diagnostic and prognostic tools, especially in population-based studies and results should therefore always be interpreted in this context. Risk of circular reasoning can also occur if the clinical diagnoses are used to validate the biomarkers and those same biomarkers are used to validate the clinical diagnoses without any consideration of the concordance between the clinical diagnoses and autopsy findings [249].

Finally, there may also be challenges associated with the transition towards earlier diagnosis as the diagnosis will rely heavily on biomarkers. As described, normal cognition is associated with deposition of the typical pathological hallmarks of AD [216], and even if these subjects would eventually have developed dementia if they had lived long enough, the underlying pathology did not affect their lifespan nor their quality of life. In such people, it is likely that a pharmacological intervention would lead to more harm (e.g. adverse events, healthcare costs etc.) than good [250]. In other words, early diagnosis may lead to over diagnosis if the underlying processes and pathways discriminating the “benign” abnormalities from the abnormalities driving the progression to dementia are not properly understood.

10

Concluding Remarks

10. Concluding Remarks

10.1 Conclusion

The results presented in this thesis provide evidence that modifiable risk factors are associated with late-life dementia and thereby add to the existing evidence from previous epidemiological studies on dementia and cognitive impairment.

In paper II and III we found that physical inactivity, a history of depression and impaired fasting glucose increased the risk of developing dementia while overweight (BMI 25–29.9), as compared with normal weight women was associated with a lower risk of dementia. Also the precursors of type 2 diabetes; impaired fasting plasma glucose and insulin resistance were associated with increased risk of developing cognitive dysfunction in the subpopulation who attended the follow-up study. This jointly suggests that a significant proportion of dementia cases in women is likely to be preventable by effective prevention and control of these modifiable risk factors.

For subjects with signs of mild objective cognitive impairment at baseline we found that the degree of impairment at baseline along with age, physical inactivity, higher level of total cholesterol and a history of depression were associated with cognitive progression over the follow-up period.

Finally we showed that serum levels of two truncated tau species, Tau-A and Tau-C, were associated with incident dementia underlining the hypothesis that these biomarkers may serve as non-invasive, affordable and widely available tools that can aid in early identification as well as guide the design of clinical trials for identification of the right patients and potentially also monitoring of treatment efficacy either alone or as part of a biomarker panel.

The findings jointly serve as an example of a first in-line screening tool for the identification of at-risk individuals which could serve two obvious needs: *i)* identifying subjects in most need of preventive interventions or *ii)* identify subjects suitable for enrollment in clinical trials of new disease-modifying interventions.

10.2 Perspectives

There has been an enormous and accelerating scientific effort in the study of dementia etiology and pathophysiology, all driven by an overarching objective to curb the epidemic. To redeem this ambitious goal there is still a need for a better understanding of the pathways leading to dementia. In LOAD for example, where a plethora of processes are speculated to be involved in the pathogenesis, we need to understand what triggers these processes and how they interact through time. This complexity will require systems-based approaches where different sources of

data (genetic, epigenetic, proteomic and environmental factors) are jointly integrated. There are data-sharing initiatives on-going that will enable more systems-based approaches in big data in the future [251]. Such systems-based approach would also aid in the identification of different phenotypes and thereby potentially promote precision medicine by the identification of patients who are in greatest need and who may benefit the most from a given treatment. An early example of a systemic approach used a biomarker-guided cluster analysis to identify five different subgroups of AD [252], subgroups that are speculated to respond differently to pharmacological treatment [253].

With the availability of an extensive amount of registry data on comorbidities, the observations and data from PERF I and PERF II and the potential for analyzing genetic material (available in the biobank) the PERF cohort is a great resource in relation to the systems-based approach. Also, there is great potential in further research on dementia risk factors for example all participants underwent whole body dual energy X-ray absorptiometry (DEXA) scanning at the baseline visit and this gives a unique possibility to study associations between body composition (bone, muscles and fat) and dementia.

The tau biomarkers and potentially other future blood-based biomarkers based on this technology (Specifically focusing on protein fragments generated by disease-specific combinations of proteins and proteases) have potential to benefit subjects with pathophysiological signs of disease. The future work should focus on validating the biomarkers in large prospective studies designed specifically for dementia. Studies should clarify the biomarker trajectories and their relation to diagnosis, prognosis and prediction but also focus on mapping the processes from the initial protein cleavage to the measurement of the fragments in blood.

The nomogram presented in this thesis require validation. First the efforts should be focused on whether a simplification of the predictive model can be done without significantly altering the predictive accuracy. Also the performance of the predictive model should be evaluated with e.g. cross-validation and subsequent validation in an independent cohort.

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Appendix

Appendix A Poster Presentations

Alzheimer Europe Conference, 2015 in Ljubljana, Slovenia.
Risk Factors for Development of Dementia: The Prospective Epidemiologic Risk Factor (PERF I) Study

Clinical Trials on Alzheimer's Disease (CTAD) Conference, 2015 in Barcelona, Spain.
Modifiable Risk Factors for Dementia: A Strategy to Counteract the Poor Prospects?

Identification of Fast Progression Dementia Phenotype: a Comparative Evaluation of Two Short Cognitive Screening Instruments

The Alzheimer's Association International Conference (AAIC), 2016 in Toronto, Canada.
Tau-C, a Caspase-3 cleaved fragment of Tau: A Serum Biomarker for Preclinical Dementia

Appendix B Additional manuscripts

I have contributed to six additional manuscripts during the PhD, which are not integrated in the thesis:

First-author manuscript

JS Neergaard, K Dragsbæk, C Christiansen, HB Nielsen, CT Workman, S Brix, K Henriksen, MA Karsdal. **Modifiable Risk Factors Promoting Neurodegeneration is associated with two novel Brain Degradation markers measured in serum.** In 1st review in Neurochemistry International, Submitted December 2016.

K Dragsbæk*, **JS Neergaard***, HB Hansen, I Byrjalsen, P Alexandersen, SN Kehlet, AC Bay-Jensen, C Christiansen, and MA Karsdal. **Matrix Metalloproteinase Mediated Type I Collagen Degradation – An Independent Risk Factor for Mortality in Women.** EBioMedicine 2015: 30;2(7):723-9.

Co-author manuscripts

K Dragsbæk, **JS Neergaard**, JM Laursen, HB Hansen, C Christiansen, H Beck-Nielsen, MA Karsdal, S Brix, and K Henriksen. **Metabolic Syndrome and Subsequent Risk of Type 2 Diabetes and Cardiovascular Disease in Elderly Women: Challenging the Current Definition**. Medicine (Baltimore) 2016;95(36):e4806.

K Dragsbæk, **JS Neergaard**, C Christiansen, MA Karsdal, H Beck-Nielsen, S Brix, and K Henriksen. **Weight Change and Risk of Hyperglycemia in Elderly Women**. Aging Clinical and Experimental Research 2017: In press

CL Bager, N Willumsen, SN Kehlet, HB Hansen, AC Bay-Jensen, DJ Leeming, K Dragsbaek, **JS Neergaard**, C Christiansen, E Høgdall, and MA Karsdal. **Remodeling of the tumor microenvironment predicts increased risk of cancer in postmenopausal women - The Prospective Epidemiologic Risk Factor (PERF I) Study**. Cancer Epidemiol Biomarkers Prev; 25(9); 1348–55.

N Willumsen, CL Bager, SN Kehlet, K Dragsbæk, **JS Neergaard**, HB Hansen, AC Bay-Jensen, DJ Leeming, A Lipton, C Christiansen and M Karsdal. **Excessive matrix metalloprotease-mediated degradation of interstitial tissue (type I collagen) independently predicts short-term survival in an observational study of postmenopausal women diagnosed with cancer**. Accepted for publication in Oncotarget January 2017