

# **Risk factors for dementia**

Lifestyle, hormones, neurochemistry, and genetics

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*In the loving memory of my brilliant grandfather, Ibrahim Najar, for his desire to always learn and study, and for his everlasting love for my wonderful grandmother,  
Dagheela Najar.*

*I will always love you.*



# ABSTRACT

**Background:** Dementia is one of the greatest global challenges today. Given the expected increase in people with dementia, it is important to study risk factors for dementia to identify individuals at increased risk in order to implement preventative strategies before dementia pathology starts to accumulate.

**Objective:** The aim of this thesis was to expand the understanding about the effects of lifestyle factors, indicators of endogenous estrogens, and genetic factors on the risk of dementia and cerebrospinal fluid (CSF) markers for Alzheimer's disease (AD).

**Method:** We used population-based samples from the Gothenburg H70 Birth Cohort Studies (H70-studies), the Prospective Population Study of Women (PPSW), and the Mayo Clinic Study of Aging (MCSA 70+ study). Information on exposures (marital status [married vs not married], cognitive and physical activity [active vs inactive], indicators of endogenous estrogen [age at menarche and menopause, reproductive period, number of pregnancies, and months of breastfeeding], and genetic factors [polygenic risk scores for AD (AD-PRSs) and *APOE* genotype]) was obtained through interviews and examinations performed by experienced health personnel. Dementia was diagnosed according to established criteria based on information from the examinations. CSF levels of A $\beta$ 42, A $\beta$ 40, P-tau, and T-tau were measured with immunochemical methods.

**Results:** In *Project I* (the H70-studies, n=913; the MCSA 70+ study, n=3,471), we found that married men had a reduced risk of dementia compared to unmarried men, while no association was found between marital status and incident dementia among women. In *Project II* (PPSW and the H70-studies, n=784), we found that midlife cognitive and physical activity were independently associated with reduced risk of late-life dementia disorders. In *Project III* (PPSW and the H70-studies, n=1,364), we found that longer reproductive period and later age at menopause were associated with increased risk of dementia and AD, particularly in those with dementia and AD onset after age 85 years. In *Project IV* (PPSW and the H70-studies, n=75), we found that longer reproductive period was associated with CSF biomarkers for AD (lower levels of A $\beta$ 42, lower ratio of A $\beta$ 42/A $\beta$ 40, and higher levels of P-tau). In *Project V* (the H70-studies, n=2,052), we found that AD-PRSs (including 39 and 57 genetic variants) and *APOE* genotype were associated with risk of dementia up to very old ages. The association between AD-PRSs and risk of dementia was particularly strong in *APOE*  $\epsilon$ 4 non-carriers.

**Conclusion:** The results from this thesis add knowledge about risk factors for dementia, and add further knowledge on the protective effects of cognitive and physical activity on risk of dementia disorders.

**Keywords:** Dementia, Alzheimer's disease, marital status, leisure time activity, menopause, polygenic risk scores, *APOE* genotype.



# SAMMANFATTNING PÅ SVENSKA

Demens är idag en av världens största folkhälsosjukdomar. Då antalet personer med demens förväntas öka de kommande åren är det viktigt att studera riskfaktorer för sjukdomen, för att kunna identifiera förebyggande strategier som kan implementeras innan sjukdomsprocesserna börjar ansamlas.

Syftet med den här avhandlingen var att öka kunskapen om hur livsstilsfaktorer, kroppseget östrogen och genetiska faktorer påverkar risken att drabbas av demens och nivåer av Alzheimers-markörer i cerebrospinalvätskan (CSV).

Vi använde oss av de populationsbaserade H70-studierna och Kvinnostudien från Göteborg och the Mayo Clinic Study of Aging (MCSA 70+ studien) från Rochester, Minnesota, USA. Information om de riskfaktorer vi studerade (civilstånd [gift vs ogift], hjärnstimulerande aktiviteter och fysisk aktivitet [aktiv vs inaktiv], indikatorer för kroppseget östrogen [ålder vid menarche och menopaus, reproduktionstid, antal graviditeter och månader av amning], och genetiska faktorer [genetisk riskpoäng för Alzheimers sjukdom och *APOE* genotypen]) inhämtades från intervjuer och hälsoundersökningar som genomfördes av sjuksköterskor och läkare. Demens diagnostiserades med hjälp av etablerade forskningskriterier baserat på information från undersökningarna. Nivåer av Alzheimers-markörer i CSV analyserades med etablerade laboriemetoder.

Sammanfattning av de viktigaste resultaten: I **Projekt I** (H70-studierna, n=913; MCSA 70+ studien, n=3,471), fann vi att gifta män hade en minskad risk att drabbas av demens i jämförelse med ogifta män, medan vi inte fann någon relation mellan civilstånd och demensrisk hos kvinnor. I **Projekt II** (Kvinnostudien och H70-studierna, n=784), fann vi att hjärnstimulerande aktivitet och fysisk aktivitet i medelåldern, var och en för sig, minskade risken för olika demenssjukdomar senare i livet. I **Projekt III** (Kvinnostudien och H70-studierna, n=1,364), fann vi att kvinnor med en längre reproduktionstid och senare menopausålder hade en ökad risk att drabbas av demens och Alzheimers sjukdom. Risken var framförallt hög för de som insjuknade i demens och Alzheimers sjukdom efter 85 års ålder. I **Projekt IV** (Kvinnostudien och H70-studierna, n=75), fann vi att kvinnor med en längre reproduktionstid hade högre nivåer av Alzheimers-markörer i CVS. I **Projekt V** (H70-studierna, n=2,052), fann vi att genetisk riskpoäng för Alzheimers sjukdom och *APOE*-genotypen var kopplade till risken att drabbas av demens upp till väldigt höga åldrar. Effekten av genetisk riskpoäng för Alzheimers sjukdom var speciellt stark hos de individer som inte var bärare av *APOE*  $\epsilon 4$  allelen.

Sammanfattningsvis bidrar resultaten från den här avhandlingen med ökad kunskap om riskfaktorer för demens, samt bidrar med ökad kunskap om hjärnstimulerade och fysisk aktivitets skyddande effekter på risken att drabbas av olika demenssjukdomar.





# LIST OF PAPERS

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

- I. **Najar J.** Aakre A.J, Vassilaki M, Wetterberg H, Rydén L, Zettergren A, Skoog I, Jack C.R, Knopman D.S, Petersen R.C, Kern S, Mielke M.M. Sex differences in the relation between marital status and dementia risk in two population-based cohorts. (*Submitted manuscript*)
- II. **Najar J.** Östling S, Gudmundsson P, Sundh V, Johansson L, Kern S, Guo X, Hällström T, Skoog I. Cognitive and physical activity and dementia: A 44-year longitudinal population study of women. *Neurology*. 2019; 92(12): e1322–e1330.
- III. **Najar J.** Östling S, Waern M, Zettergren A, Kern S, Wetterberg H, Hällström T, Skoog I. Reproductive period and dementia: A 44-year longitudinal population study of Swedish women. *Alzheimer's Dement*. 2020; 16(8): 1153–1163.
- IV. **Najar J.** Hällström T, Zettergren A, Johansson L, Joas E, Mellqvist Fässberg M, Zetterberg H, Blennow K, Kern S, Skoog I. Reproductive period and preclinical cerebrospinal fluid markers for Alzheimer's disease. (*Accepted for publication in Menopause*)
- V. **Najar J.** van der Lee S.J, Joas E, Wetterberg H, Hardy J, Guerreiro R, Bras J, Waern M, Kern S, Zetterberg H, Blennow K, Skoog I, Zettergren A. Polygenic risk scores for Alzheimer's disease are related to dementia risk in APOE  $\epsilon$ 4 negatives. *Alzheimer's Dement: Diagnosis, Assessment & Disease Monitoring*. 2021; 13(1): e12142.

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# ABBREVIATIONS

A $\beta$	Amyloid- $\beta$
A $\beta$ 40	40 aminoacid amyloid- $\beta$ peptides
A $\beta$ 42	42 aminoacid amyloid- $\beta$ peptides
AD	Alzheimer's disease
AD-PRS	Polygenic risk score for Alzheimer's disease
APA	The American Psychiatric Association
<i>APOE <math>\epsilon</math>4</i>	$\epsilon$ 4 allele of <i>apolipoprotein E</i> gene
<i>APOE <math>\epsilon</math>3</i>	$\epsilon$ 3 allele of <i>apolipoprotein E</i> gene
<i>APOE <math>\epsilon</math>2</i>	$\epsilon$ 2 allele of <i>apolipoprotein E</i> gene
APP	Amyloid precursor protein
<i>APP</i>	<i>Amyloid precursor protein</i> gene
BMI	Body mass index
CI	Confidence interval (95%)
CSF	Cerebrospinal fluid
CT	Computed tomography
CVD	Cerebrovascular disease
DLB	Dementia with Lewy bodies
DSM	Diagnostic and Statistical Manual of Mental Disorders
DSM-I	Diagnostic and Statistical Manual of Mental Disorders, 1 <sup>st</sup> Edition
DSM-III-R	Diagnostic and Statistical Manual of Mental Disorders, 3 <sup>rd</sup> Edition-Revised
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4 <sup>th</sup> Edition
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders, 4 <sup>th</sup> Edition- Text revision
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5 <sup>th</sup> Edition
ECG	Electrocardiogram
EOAD	Early onset Alzheimer's disease (onset $\leq$ 65 years)
FSH	Follicle stimulating hormone
FTD	Frontotemporal dementia
GnRH	Gonadotropin-releasing hormone
GWAS	Genome wide association study
H70-studies	The Gothenburg H70 Birth Cohort Studies
HR	Hazard ratios
HT	Hormone therapy
ICD	International Classification of Diseases
ICD-10	International Classification of Diseases, 10 <sup>th</sup> revision
IQR	Inter-quartile range

LD	Linkage disequilibrium
LH	Luteinizing hormone
LOAD	Late onset Alzheimer's disease (onset >65 years)
MAF	Minor allele frequency
MCSA 70+ study	The Mayo Clinic Study of Aging
MMSE	Mini-Mental State Examination
MN	Minnesota
MRI	Magnetic resonance imaging
N	Sample size
NINCDS-ADRDA	The National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association
NINDS-AIREN	The National Institute of Neurological Disorders and Stroke– Association Internationale pour la Recherche et l'Enseignement en Neurosciences
OC	Oral contraceptives
PC	Principal component
PD	Parkinson's disease
PDD	Parkinson's disease dementia
PET	Pittsburgh compound B-Positron emission tomography scans
PPSW	The Prospective Population Study of Women
PRS	Polygenic risk score
<i>P</i>	<i>P</i> value
<i>PSEN1</i>	<i>Presenilin 1</i> gene
<i>PSEN2</i>	<i>Presenilin 2</i> gene
P-tau	(Hyper)phosphorylated tau
QC	Quality control
RSQ	Imputation R <sup>2</sup> (Imputation quality)
SD	Standard deviation
SES	Socioeconomic status
SNP	Single nucleotide Polymorphism
TIA	Transient ischemic attack
T-tau	Total tau
VaD	Vascular dementia
VCI	Vascular cognitive impairment
WHO	World Health Organization
WHR	Waist-hip-ratio
$\alpha$	Alpha
$\beta$	Beta



# DEFINITIONS IN SHORT

## Cognitive domains

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Agnosia	Inability to process sensory (visual) information.
Aphasia	Inability to comprehend or formulate language.
Apraxia	Inability to plan and perform motor movements.
Executive function	Skills including working memory, self-control, flexible thinking, and planning, used in our every-day-life.

## Genetics

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Autosomal dominant disease	One copy of the mutation is needed to cause the disease
Autosomal recessive disease	Two copies of the mutation is needed to cause the disease
Genetic pleiotropy	One genetic variant is associated with different phenotypes (e.g., diseases)
Genome-wide significance	In genome-wide association studies (GWAS), over hundreds of thousands of tests are performed, increasing the likelihood to find one or more false positive associations. Therefore, based on the assumption of 1,000,000 independent associations, the significance level used in many GWASs is $P$ value $< 5 \times 10^{-8}$ .
Genotype imputation	The process of estimating genotypes using large reference panel of human haplotypes such as the Haplotype Reference Consortium (HRC).
Genotyping	Genotyping is a laboratory process to determine genetic variants of an individual. If the purpose is to examine many different and previous identified genetic variants at once, genotyping chips can be used. In this thesis, the Neurochip (Illumina) array was used.
LD clumping	Extracting only one representative SNP per region of LD.
Linkage disequilibrium	Linkage disequilibrium (LD) refers to the linkage of two genetic markers on a population level and describe the degree of which an allele of a SNP is inherited with an allele of another SNP.
Meiosis	The process of cell division of germ cells to produce gametes (e.g., sperm or egg cells).
Population stratification	As a result of assortative mating (i.e., non-random mating) between individuals, there are differences in allele frequencies between ethnical groups within a population, referred to as population stratification.





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# 1 INTRODUCTION

## 1.1 DEMENTIA PREVALENCE AND INCIDENCE

Dementia is one of the greatest global challenges today.<sup>1</sup> The estimated number of people with dementia is 47 million globally.<sup>1</sup> However, due to the increasing number of people aged 65 years or older worldwide, the amount of people with dementia is expected to triple by 2050.<sup>1</sup> In Sweden, the number of people with dementia is approximately 130,000–150,000.<sup>2</sup>

Dementia prevalence (i.e., the proportion of people with the disease in a defined population at a specific time) increases exponentially with age, doubling every five years after the age of 65.<sup>3</sup> A meta-analysis reported that the age-specific prevalence for those aged 60 years and older was 5–7% in most world regions, but higher in Latin America (8.5%) and lower in sub-Saharan Africa regions (2–4%).<sup>4</sup> Similar to dementia prevalence, incidence (i.e., the proportion of new cases over a specific period in a defined population) also doubles every five years after the age of 65.<sup>3</sup> The annual age-specific rates of dementia ranged from 0.1% in those aged 60–64 years to 8.7% in those aged 95 years or older.<sup>5</sup>

It should be noted, however, that in recent years several studies reported a decline in the age-specific prevalence and incidence of dementia in Europe and USA.<sup>6–12</sup> One recent study, including pooled data from Europe and United States, reported a decline in dementia incidence of 7–19% per calendar decade.<sup>12</sup> This positive trend could be explained by improved control of cardiovascular risk factors, stroke, and increased educational attainment in more recently born birth cohorts.<sup>8,10</sup>

### 1.1.1 SEX DIFFERENCES IN DISEASE BURDEN

Several studies report a higher dementia prevalence in women compared to men, particularly at higher ages.<sup>4,13–17</sup> However, regarding sex differences in incidence rates, studies show conflicting results. Two studies from Sweden,<sup>18,19</sup> and one study including pooled data from seven European countries,<sup>20</sup> reported sex differences in dementia incidence, especially after age 85 years. Further, a large study from the 10/66 study (population-based study from urban sites in Cuba, the Dominican Republic, and Venezuela, and rural and urban sites in Peru, Mexico, and China) reported a higher dementia incidence in women than in men.<sup>21</sup> In contrast, the Framingham Study,<sup>22</sup> a study from Rochester, Minnesota,<sup>23</sup> the 90+ Study,<sup>24</sup> and two meta-analyses<sup>5,25</sup> did not find any sex difference in dementia incidence. However, the two latter meta-analyses reported an increased risk of Alzheimer's disease (AD) among women compared to men,<sup>5,25</sup> particularly after age 85 years.<sup>25</sup> In line with these findings, studies conducted

in Europe report a higher incidence of AD among women than in men, particularly after age 80–85 years.<sup>18,19,26</sup>

If women are at increased risk of dementia (particularly AD) compared to men, what could be possible explanations? First, the higher dementia and AD prevalence among women than in men could be explained by differences in survival, as women live longer than men on average,<sup>27</sup> and also live longer with dementia compared to men.<sup>3,4,27</sup> Second, the populations examined to this point grew up at a time when women had less education.<sup>3</sup> Third, the apolipoprotein E (*APOE*) genotype, equally common in both men and women, may have a more harmful effect on dementia risk among women compared to men.<sup>3,28</sup> Fourth, men die earlier of vascular causes compared to women, creating a healthier survival cohort of men with less vascular risk factors in older ages.<sup>3</sup> Fifth, risk factors restricted to women (i.e., sex-specific risk factors), such as age at menopause, reproductive period, number of childbirths, and hormone therapy (HT) may affect cognition at later stages of life.<sup>28</sup>

## 1.2 DIAGNOSTIC CRITERIA OF DEMENTIA

Dementia is a clinical syndrome characterized by a progressive decline in cognitive abilities and activities of daily living, and is a major reason for disability and dependence.<sup>4</sup> The diagnosis of dementia relies on a set of diagnostic criteria. The two diagnostic systems used in this thesis are the Diagnostic and Statistical Manual of Mental Disorders (DSM), produced by the American Psychiatric Association (APA), and the International Classification of Diseases (ICD), produced by the World Health Organization (WHO). APA published the first edition of the DSM (DSM-I) in 1952.<sup>29</sup> During the last 30 years, subsequent revisions have been published (DSM-III-R<sup>30</sup>, DSM-IV<sup>31</sup>, and DSM-5<sup>32</sup>) with important changes, described in more detail below. ICD was originally introduced in 1900 to classify causes of death, and has since then gone through several revisions.<sup>3,33</sup> In 1993, the tenth edition of ICD (ICD-10) was introduced.<sup>3,34</sup>

The diagnostic systems (DSM-III-R, DSM-IV, DSM-5 and ICD-10) differ somewhat in their diagnostic criteria for dementia.<sup>30-32,34</sup> In the DSM-III-R, DSM-IV, and the ICD-10 memory impairment is mandatory; DSM-III-R require impairment in both short- and long-term memory, while DSM-IV and ICD-10 only require impairment in either memory domain.<sup>30,31,34,35</sup> In contrast, in DSM-5, memory impairment is not required for dementia diagnosis. Further, DSM-5 require substantial decline in only one out of six cognitive domains for a dementia diagnosis, while DSM-III-R, DSM-IV, and ICD-10 require memory impairment and decline in one other cognitive domain.<sup>32,36</sup> Moreover, in addition to memory impairment and decline in other cognitive domains, ICD-10 also require personality symptoms (emotional lability, irritability, apathy, or coarsening of social behaviour) for diagnosis of dementia, while

personality symptoms are not mandatory in DSM-III-R or DSM-5, and are not included in the DSM-IV criteria.<sup>30-32,34,35</sup> A more detailed description of the diagnostic criteria of dementia according to DSM-III-R, DSM-IV, DSM-5, and ICD-10 are shown in the Appendix 1 and 2.

## 1.3 DEMENTIA SUBTYPES

There are different forms of dementia that have similar clinical presentations but are distinguished based on etiology. The main dementia subtypes discussed in this thesis are Alzheimer's disease (AD), vascular dementia (VaD), and vascular cognitive impairment (VCI).

### 1.3.1 ALZHEIMER'S DISEASE

Dr. Alois Alzheimer, a German psychiatrist, first recognized AD (later named after him) in the beginning of the twentieth century.<sup>3</sup> The first case he described was Aguste Deter, a 51 year-old woman who presented with symptoms of psychosis (jealousy of her husband and paranoia) and cognitive impairment (progressive memory loss and disorientation, aphasia, and alexia). She lived for four and a half years after her first symptoms began, and was in the end lying in fetal position, incontinent, with bedsores.<sup>3,37</sup> In 1907, he published the first case report of Aguste Deter.<sup>3,37,38</sup> In the case report, he described that thick fibrils, in bundles, were found inside of neurons, and that adjacent cells had similar findings.<sup>3,37</sup> These fibrils sometimes also occurred outside of degenerated neurons.<sup>3,37</sup> He also described numerous small "miliary foci" (amyloid plaques) spread throughout the cortex, causing cell death and neurodegeneration.<sup>3,37</sup>

AD is the leading cause of dementia, accounting for 50–60% of all dementia cases.<sup>3</sup> The prevalence of AD doubles every 4.3 years, with an age-adjusted rate of 19.2 per 1000 person-years.<sup>39</sup> The clinical manifestation of AD includes insidious onset of a progressive decline in memory and other cognitive functions, such as aphasia, apraxia, and agnosia.<sup>40,41</sup> A detailed description of AD is found in Appendix 3.

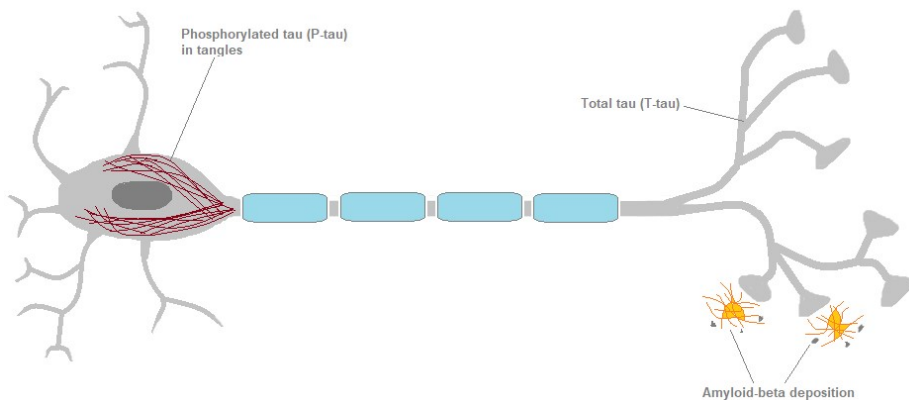
Depending on age at dementia onset, AD is classified as early-onset AD (EOAD) or late-onset AD (LOAD).<sup>42</sup> EOAD is defined as AD with a clinical onset before or at age 65 years, while LOAD is defined as AD with clinical onset after age 65 years.<sup>42</sup> Although LOAD accounts for the vast majority of all AD cases, EOAD accounts for approximately 10% of all AD cases and is the most common cause of early-onset dementia.<sup>43</sup>

### 1.3.1.1 PATHOGENESIS OF ALZHEIMER'S DISEASE

The pathological features of EOAD and LOAD are mainly the same, and even though some studies have reported that patients with EOAD present with a larger neurological burden and a more widespread pathology outside the medial temporal lobe, at the end-stage of the disease it is hard to distinguish the two types of AD neuropathologically.<sup>43</sup>

In the brain, AD is characterized by the aggregation of amyloid- $\beta$  ( $A\beta$ ) into plaques, hyperphosphorylation and aggregation of tau proteins into tangles, as well as atrophy due to neurodegeneration.<sup>44</sup> AD pathology affects the medial temporal lobe structures and cortical areas of the brain, as well as neurons and synapses.<sup>44</sup>

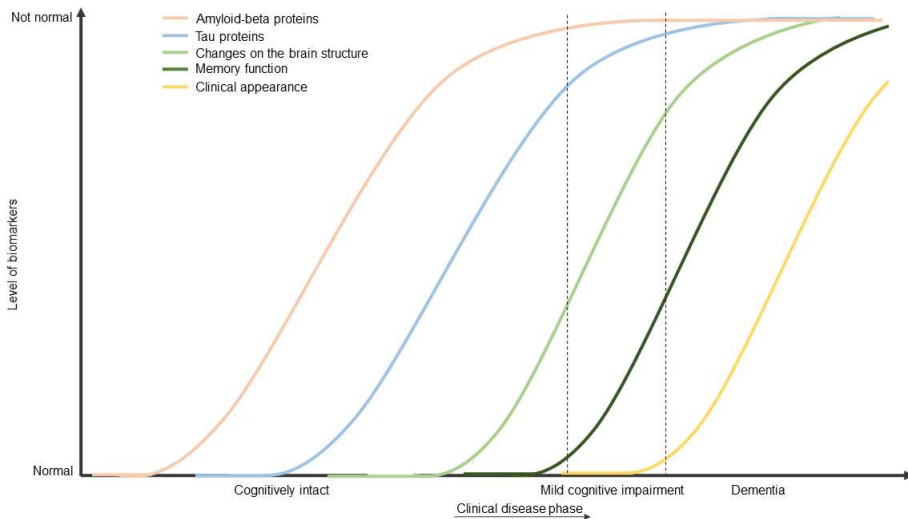
The principal theory for the cause of AD is the amyloid cascade hypothesis, suggesting an imbalance between production and clearance of  $A\beta$  in the brain that leads to neuronal degeneration and dementia.<sup>44,45</sup>  $A\beta$  is produced through the metabolism of amyloid precursor protein (APP).<sup>44</sup> APP is a transmembrane protein, containing a large N-terminal extracellular tail that can be processed along two main pathways (non-amyloidogenic and amyloidogenic pathways).<sup>44</sup> The non-amyloidogenic pathway, include the cleavage of APP by  $\alpha$ -secretase within the  $A\beta$  domain, releasing a large soluble fragment of APP.<sup>44</sup> The remaining C-terminal fragment of APP is further cleaved by the  $\gamma$ -secretase, while the intracellular domain is metabolized in the cytoplasm.<sup>44</sup> The cleavage of APP by  $\alpha$ -secretase, within the  $A\beta$  domain, prevents deposition of  $A\beta$ .<sup>44</sup> In contrast, the amyloidogenic pathway, caused by  $\beta$ -secretase cleavage of APP just before the  $A\beta$  domain and further by the  $\gamma$ -secretase, result in an increased deposition of free 40 ( $A\beta_{40}$ ) or 42 ( $A\beta_{42}$ ) aminoacid  $A\beta$  peptides, with the latter  $A\beta$  most prominent in AD (Figure 1).<sup>44,46</sup>



**Figure 1.** Illustration of a neuron affected by Alzheimer's disease pathology. Source: Original by the author, based on Blennow and Zetterberg 2018.<sup>47</sup> Amyloid- $\beta$  is accumulated outside the neuron. Tau are axonal proteins. P-tau reflects the amount of phosphorylated tau, which is found in tangles within the cell. T-tau reflects level of neurodegeneration.

Support for the amyloid cascade hypothesis include mutations found in families with AD, the *amyloid precursor protein* gene (*APP*), *presenilin 1* (*PSEN1*) and *presenilin 2* (*PSEN2*) genes (described in more detail on page 16), which have been found to accelerate A $\beta$ 42 production.<sup>46,48,49</sup> Mutations in *APP* affect the processing of the encoded protein and increase the self-aggregation of A $\beta$  into amyloid fibrils, while mutations in *PSEN1* and *PSEN2* impair the cleavage of A $\beta$  mediated by  $\gamma$ -secretase, which result in an increase in the A $\beta$ 40:A $\beta$ 42 ratio (either through an increase of A $\beta$ 42 levels, or a decrease in A $\beta$ 40 levels).<sup>45,46</sup>

Tau proteins are axonal proteins that promote microtubule assembly and stability. In AD, tau proteins are hyperphosphorylated into insoluble fibrils in tangles (Figure 1).<sup>44</sup> The phosphorylation of tau (P-tau) is regulated by the balance between multiple kinases and phosphates and causes disassembly of microtubules, impaired axonal transport, and impaired neuronal and synaptic function.<sup>44</sup> It is hypothesized that processes of AD start decades before clinical symptoms appear (i.e., preclinical AD),<sup>50</sup> where the toxic accumulation of A $\beta$  induce hyperphosphorylation and aggregation of tau (Figure 2).<sup>50</sup> At later stages brain structures are affected (e.g., atrophy visible on Magnetic resonance imaging [MRI]) and clinical symptoms appear.<sup>50</sup>



**Figure 2.** The Alzheimer's disease pathological cascade. Source: Jack et al. 2010,<sup>50</sup> with permission from Elsevier, adapted by the author.<sup>50</sup>

In cerebrospinal fluid (CSF), lower levels of A $\beta$ 42 and higher levels of P-tau reflect brain pathology of AD, while high CSF levels of total tau (T-tau) reflect neurodegeneration and is not a specific biomarker of AD.<sup>51</sup> A $\beta$ 40 is thought to serve as an indicator of total A $\beta$  levels and the ratio of CSF A $\beta$ 42 and A $\beta$ 40 has shown to

improve the diagnostic accuracy for AD.<sup>51</sup> Interestingly, pathological markers for AD are common in cognitively normal older individuals, with 46% having either CSF amyloid or tau-pathology at age 70 years.<sup>52</sup>

Evidence also point to a sex difference in levels of AD pathology, with higher levels of tau-pathology in women than in men,<sup>53</sup> particularly downstream the trajectory of AD pathogenesis.<sup>54,55</sup> More tau pathology (P-tau and T-tau) was observed in female *APOE*  $\epsilon 4$  carriers than in male carriers,<sup>55</sup> and greater entorhinal tau pathology was found in women with a higher amyloid burden, compared to men with high amyloid burden.<sup>54</sup> Further, in an autopsy study, women with at least one *APOE*  $\epsilon 4$  allele had more neurofibrillary tangle and amyloid plaque neuropathology compared to men.<sup>56</sup>

Moreover, based on the genetic architecture of AD (described in more detail on page 17), the immune response, cholesterol and lipid metabolism, and endosomal-vesical recycling, have been suggested as potential underlying mechanisms of the disease.<sup>57</sup> It has also been recognized that patients with LOAD, especially in older ages, present with multiple pathologies<sup>58,59</sup> such as vascular pathology,<sup>60</sup> Lewy body pathology,<sup>61</sup> and hyperphosphorylated transactive response DNA-binding protein 43 (TDP-43).<sup>61-64</sup>

### 1.3.2 VASCULAR DEMENTIA AND VASCULAR COGNITIVE IMPAIRMENT

Thomas Willis was among the first to describe the relationship between stroke (hemiplegia) and cognitive impairment.<sup>9</sup> In 1672, he wrote that “I have observed in many cases that when, the brain being indisposed, they have been distempered with dullness of mind and forgetfulness, and then afterward with a stupidity and foolishness, they would afterward have fallen into a Palsie, which I often did predict”.<sup>9,65,66</sup> Further, in 1910, Emil Kraepelin, a German psychiatrist, published one of the first descriptions of arteriosclerosis and dementia in old age,<sup>67</sup> based on previous findings by Maurice Klippel, Otto Binswanger, and Alois Alzheimer.<sup>9</sup> However, it took another half a century to further refine the definition of dementia caused by cerebrovascular disease (CVD). In 1970, with the introduction of computed tomography (CT), and MRI another decade later, cerebral atrophy, infarcts, and white matter hyperintensities (WHM), were identified as important causes of dementia.<sup>9</sup>

Vascular dementia (VaD) is considered the second most common cause of dementia, accounting for approximately 15–30% of all dementia cases.<sup>68</sup> The prevalence of VaD doubles every 5.3 years, with an age-adjusted rate of 14.6 per 1,000 person-years.<sup>39</sup> However, the improvement in control of cardiovascular risk factors and stroke might have resulted in an age-specific decline in the risk of developing VaD.<sup>8,9,69</sup>

The clinical manifestation of VaD more often include an abrupt onset and a stepwise cognitive decline caused by a cerebral insult.<sup>3</sup> A detailed description of VaD is found in Appendix 4.

During the last two decades, the concept of VaD (mainly based on stroke and the multi-infarct model) has been challenged.<sup>70</sup> In addition to VaD, the term vascular cognitive impairment (VCI) was proposed as an umbrella term to describe cognitive impairment (ranging from subjective memory impairment to dementia) caused by vascular brain pathologies, such as infarcts and WMHs, and mixed pathologies (e.g., AD pathology), where VaD denote a subgroup of patients with dementia caused more exclusively of CVD.<sup>39,68</sup> In contrast to the prevalence of VaD, if individuals with mixed dementia pathologies and with WHMs are considered, VCI accounts for between 50% and 70% of all dementia cases.<sup>68</sup>

### 1.3.2.1 PATHOGENESIS OF VASCULAR DEMENTIA

The heterogeneity of VaD has made it difficult to understand the underlying cause, which most often has been considered sporadic and related to cardiovascular risk factors.<sup>71</sup> However, the pathophysiological link between cardiovascular risk factors and cognitive impairment are still undetermined.<sup>9</sup> One theory is that cardiovascular risk factors not only cause dementia through CVD, but also contribute to neurodegeneration (e.g., via changes in blood pressure and cerebral perfusion).<sup>9</sup> Another theory is that cardiovascular risk factors and dementia share genetic risk factors.<sup>9</sup> Further, genetic variants associated with VaD also suggest that immune dysfunction could contribute to the disease pathogenesis.<sup>71</sup>

### 1.3.3 OTHER DEMENTIA SUBTYPES

Among other dementia subtypes are frontotemporal dementia (FTD), dementia with Lewy bodies (DLB), Parkinson's disease (PD) dementia (PDD), and alcohol dementia.

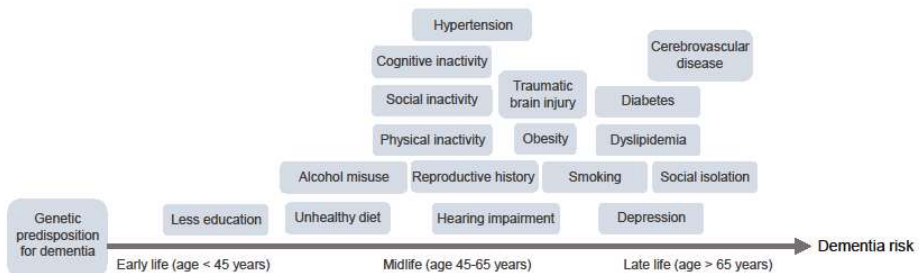
FTD comprises several syndromic variants characterized by degeneration of the frontal and anterior temporal lobes, resulting in the progression of behavioral and personality change and/or language impairment.<sup>32,72,73</sup> The prevalence of FTD ranges between 2–10 per 100,000 and is a common cause of dementia with early onset (before age 65 years).<sup>32</sup>

DLB is characterized by brain deposit of  $\alpha$ -synuclein (Lewy bodies).<sup>74</sup> The clinical manifestation includes a progressive cognitive decline (complex attention and executive function), complex visual hallucinations, symptoms of rapid eye movement (REM) sleep behavior disorder, and spontaneous parkinsonism (e.g., hypo- or bradykinesia [i.e., slowness of movement], rigidity [i.e., increased muscle tone causing stiffness and resistance to limb movement], and tremor of the hand).<sup>32</sup> PDD is also

characterized by deposit of Lewy bodies in the brain and the clinical manifestation of PDD is similar to symptoms of DLB.<sup>74</sup> However, the main clinical distinction between PDD and DLB is timing of the extrapyramidal symptoms; in DLB the Parkinsonism is presented after onset of cognitive impairment, while the Parkinson's disease has to precede onset of dementia in PDD.<sup>32,74</sup> DLB and PDD are more uncommon forms of dementia, accounting for less than 5% in the general population.<sup>32</sup> However, autopsy studies report that Lewy bodies are present in 20–35% of dementia cases.<sup>75</sup> Further, among individuals with PD, approximately 75% develop dementia during the course of their disease.<sup>32</sup>

## 1.4 RISK FACTORS FOR DEMENTIA

The accumulation of factors across the life span, from time of conception to very old age, affect the individual's risk of developing dementia.<sup>1,3,76</sup> So far, a wide range of factors have been identified for dementia, such as environmental factors (e.g. low educational attainment), lifestyle factors (e.g. social engagement, marital status, smoking status, alcohol consumption, diet, and cognitive and physical activity), cardiovascular risk factors (e.g., hypertension, diabetes, obesity, and stroke), psychiatric disorders (e.g., depression), hearing loss, traumatic head injury, and genetic factors.<sup>3,77</sup> Evidence suggest that risk factors for dementia may be important during different periods of the life course (Figure 3).<sup>77</sup> For example, risk of dementia is, to a varying extent, determined at the time of conception through the genetic architecture of the individual.<sup>78,79</sup>



**Figure 3.** Illustration of the complex interaction between risk factors for dementia accumulated at different periods during the life course. Source: Original by the author, based on Livingston et al. 2017 & 2020, Borenstein and Mortimer 2016, and Kivipelto et al. 2013.<sup>1,3,77,80</sup>

Further, the protective effect of education may be most important in early life (before 20 years of age), during the most significant period of brain plasticity.<sup>77</sup> However, educational attainment in early life may also increase the likelihood of lifelong cognitive activation.<sup>3</sup> In addition, midlife, defined as age 45–65 years, is a crucial time for dementia prevention, as many risk factors for dementia start to accumulate during this time.<sup>77,80</sup> Further, female reproductive history (e.g., age at menopause and number



of pregnancies and childbirths) are suggested to affect women's cognitive health.<sup>28</sup> Moreover, risk factors such as cerebrovascular disease, depression, smoking, and diabetes, affect dementia risk later in life (after age 65 years).<sup>77,80</sup> Evidence also suggest that the multifactorial etiology of dementia is a result of the complex interactions between both genetic and non-genetic factors accumulated during the life course.<sup>80</sup>

The focus of this thesis was to study the relationship between lifestyle factors (particularly marital status, and cognitive and physical activity), hormonal factors (particularly indicators of endogenous estrogens), and genetic factors and risk of dementia.

### 1.4.1 LIFESTYLE FACTORS AND DEMENTIA

Lifestyle factors include behaviors and habits that affect the health of an individual, such as smoking status, alcohol consumption, dietary patterns, social engagements, marital status, and leisure time cognitive and physical activity.

As aforementioned, several lifestyle factors have been associated with risk of dementia. For example, smoking has been associated with increased risk of VaD, independent of cardiovascular risk factors and stroke.<sup>1,3</sup> Several studies also report a higher risk of AD among smokers (current and former) compared to non-smokers, particularly in men.<sup>3</sup> Further, excessive alcohol consumption can cause cognitive dysfunction due to thiamine (vitamin B<sub>1</sub>) deficiency seen in Wernicke-Korsakoff syndromes and also alcohol dementia in the absence of thiamine deficiency.<sup>3</sup> In addition, the Lancet Commission 2020 reported that individuals consuming more than 168 g of alcohol per week had an increased risk of all-cause dementia, compared to lighter drinkers.<sup>77</sup> Moreover, dietary patterns, such as the Mediterranean diet (MD), the Dietary Approaches to Stop Hypertension diet (the DASH diet), and the Mediterranean-DASH Intervention for Neurodegenerative Delay diet (the MIND diet), have been examined in relation to dementia and AD risk. The MD is defined by a high intake of unrefined cereals, fruits- and vegetables, legumes, olive oil, and a moderate intake of dairy products and alcohol, and a low intake of meat.<sup>3</sup> The DASH diet is defined by a high intake of fruits- and vegetables, low-fat dairy products, nuts and legumes, whole grains, and a low intake of salt, sweetened beverages, and red/processed meat, while the MIND diet is regarded as a hybrid of the MD and the DASH diet.<sup>3</sup> The Rush Memory and Aging Project (MAP) comparing the MIND diet to the MD and DASH diet, reported that high adherence to all three diets may reduce risk of AD, while also a moderate adherence to the MIND diet was sufficient to reduce AD risk.<sup>81</sup>

The association between other lifestyle factors, such as social engagements, marital status, and leisure time cognitive and physical activity, and risk of dementia are discussed in more detail below.

#### 1.4.1.1 SOCIAL ENGAGEMENT, MARITAL STATUS AND DEMENTIA

Social engagement has a broad definition that range from socially stimulating activities (e.g., club membership and church activities), to social networks (e.g. relatives, neighbors, and other contacts), marital status, and living situation (i.e., living alone, with a spouse, or another individual). Longitudinal population-based studies have reported an association between social activity and reduced risk of dementia.<sup>82-84</sup> In contrast, one longitudinal population-based study did not find an association between midlife social engagements and incident dementia.<sup>85</sup> Instead, the study reported an increased risk of dementia in those with decreasing level of social engagement in late-life, which the authors concluded could be due to early signs of dementia and thus a result of reverse causation.<sup>85</sup>

Further, living alone, not being married or in a relationship has also been associated with increased risk of cognitive decline,<sup>86</sup> all-cause dementia,<sup>84,87-93</sup> and AD.<sup>94,95</sup> On the contrary, the population-based PAQUID study did not find an effect of marital status and size of social network on risk of dementia and AD.<sup>96</sup> However, the study reported that participants who felt satisfied with their relationship, and who received more support than they gave over their lifetime, had a reduced risk of dementia and AD,<sup>96</sup> which could suggest that quality of social interaction may be more important than quantity. In line with these findings, the population-based Kungsholmen study reported that the increased risk of dementia in individuals who lived alone with no social contacts did not remain for those who experienced infrequent social contact as satisfying.<sup>89</sup>

Moreover, systematic reviews and meta-analyses have reported an association between poor social engagement and increased risk of dementia,<sup>97,98</sup> which was mainly driven by marital status (i.e., being unmarried) and having a poor social network.<sup>97</sup> In line with these findings, a recent systematic review and meta-analysis reported that being married was associated with reduced risk of dementia, compared to being widowed or lifelong single.<sup>99</sup>

Previous studies also report that marital status is associated with other comorbidities among older adults, such as depression,<sup>100</sup> cardiovascular disease,<sup>101</sup> and mortality,<sup>102</sup> and that the effect of marital status on health may be modified by sex. Two studies reported that single, widowed, and divorced men were at increased risk of depression compared to women.<sup>103,104</sup> Another study reported an increased risk of hypertension

and death in men who had never been married compared to married men, while no association was observed in women.<sup>105</sup> However, few studies have examined the modifying effect of sex on the association between marital status and risk of dementia. A population-based study, the Health and Retirement Study, reported higher odds of dementia in divorced, separated, and widowed men, compared to women.<sup>91</sup> Another register-based study from Sweden reported that divorced men had a higher risk of dementia compared to divorced women. However, in the latter study, the sex difference did not remain significant in the fully adjusted model.<sup>92</sup>

#### 1.4.1.2 COGNITIVE AND PHYSICAL ACTIVITY AND DEMENTIA

In dementia research, cognitive activity is referred to as mentally stimulating leisure time activities, such as reading a book, writing, playing an instrument, doing crossword puzzles, or mentally stimulating activities as part of the occupation. Physical activity has also a broad definition in dementia research ranging from physically stimulating leisure time activity, such as walking, cycling, swimming, housework, to physically stimulating activity during occupation, and to more regular and intense training.

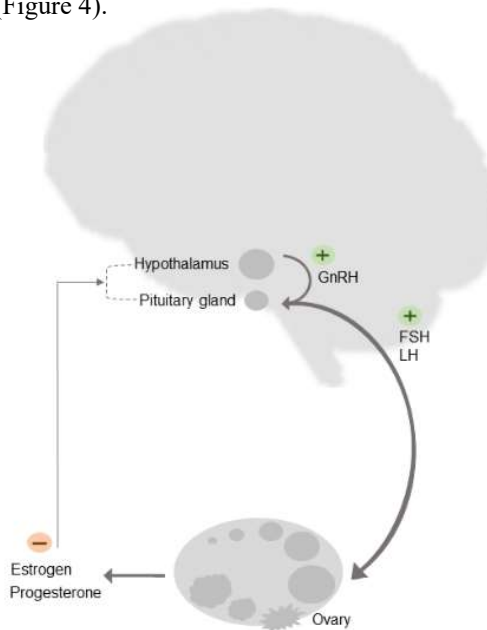
Prospective studies have reported that cognitive<sup>83,106-108</sup> and physical activity<sup>106,109-111</sup> are associated with reduced risk of dementia and AD, while other studies have failed to support these findings.<sup>83,107,108</sup> However, most studies have a high mean age at baseline (65 years or older), when information on activity levels was obtained, and relatively short follow-ups (less than 7 years). As aforementioned, it is suggested that dementia pathology, particularly AD, start to accumulate decades before clinical symptoms appear.<sup>51</sup> Thus, low levels of cognitive and physical activity may be symptoms of preclinical dementia in studies including individuals aged 65 years or older at baseline and with short observation periods.

Among studies with longer observation periods (17 to 44 years), cognitive<sup>82,112</sup> and physical<sup>82,113-118</sup> activity, and physically fitness,<sup>119</sup> has been associated with reduced risk of dementia and AD. However, in contrast, three longitudinal studies did not find a relation between physical activity and dementia risk.<sup>112,120,121</sup> Nevertheless, the positive effects of cognitive and physical exercise on cognition are supported by randomized controlled trials (RCTs). One recent large and comprehensive systematic review of RCTs, including individuals aged 50 years or older, reported that physical exercise interventions (aerobic exercise, resistance training, and tai chi), for at least 45 minutes at a moderate to vigorous intensity, improved cognitive function regardless of cognitive status.<sup>122</sup> The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER), reported positive effects of a 2-year multi-domain intervention (diet, exercise, cognitive training, and vascular disease monitoring) on cognition.<sup>123</sup> The Advanced Cognitive training for Independent and Vital Elderly (ACTIVE) study reported that 10–14 weeks of organized cognitive training improved cognitive function in older adults five and ten years later compared

to non-trained individuals.<sup>124</sup> However, in 2019 WHO published “The risk reduction guidelines for cognitive decline and dementia” including recommendations for preventive strategies against cognitive decline and dementia.<sup>125</sup> In the report, evidence for the beneficial effects of physical activity on dementia risk was rated as “moderate” and evidence for the beneficial effects of cognitive activity was rated “very low to low”.<sup>125</sup>

## 1.4.2 ESTROGEN AND DEMENTIA

Estrogens are steroid hormones synthesized by the enzyme *aromatase* in the ovaries in fertile women, and by the placenta during pregnancies. Estrogen is also synthesized in other tissues such as adipose and skin tissues, bone, and in the brain.<sup>126</sup> In postmenopausal women, the principal sites of estrogen synthesis are in the adipose and skin tissues.<sup>126</sup> In men, estrogen formation occur in the testicles as well as in the skin and adipose tissues.<sup>126</sup> The production of estrogen in the ovaries are controlled by the hypothalamic-pituitary-ovarian axis (Figure 4). The hypothalamus signals the anterior pituitary gland, through the gonadotropin-releasing hormone (GnRH), to release follicle stimulating hormone (FSH) and luteinizing hormone (LH). In turn, FSH and LH stimulate the ovaries to produce sex hormones, estrogens and progesterone. Finally, estrogen performs a negative feedback loop on the hypothalamus, decreasing the production of GnRH (Figure 4).



**Figure 4.** The hypothalamic-pituitary-ovarian axis. Source: Original by the author. The figure is a simplified illustration of the hypothalamic-pituitary-ovarian axis.

In the body, estrogen affects most organ systems.<sup>127</sup> In the brain, estrogen regulates glucose transport, aerobic glycolysis, mitochondrial function to generate adenosine triphosphate (ATP), and protects against DNA damage caused by oxidative stress.<sup>127</sup> Estrogen also affects brain regions important for learning and memory, including the prefrontal cortex, hippocampus, amygdala, and posterior cingulate cortex.<sup>128</sup>

In women, there are three forms of estrogen circulating the body: estrone, estriol, and estradiol.<sup>127</sup> The latter form of estrogen, 17 $\beta$ -estradiol, is the principal and most potent form of estrogen in fertile women, while estrone is the principal estrogen in postmenopausal women.<sup>127,129</sup> In this thesis, the term *estrogens* will be used to denote the different forms of estrogens circulating the female body.

Endogenous (i.e., from inside the body) estrogen levels change during the woman's life course due to certain sex-specific events. The female fertility starts with the menarche, defined as the first menstruation, and ends with the menopause, defined as one year without menstruation. This period is also referred to as the reproductive period. Pregnancies involve higher levels of estrogens, while estrogen levels drop in the postpartum phase, particularly among breastfeeding women. Thus, age at menarche and menopause, reproductive period, number of pregnancies, and months of breastfeeding could be regarded as indicators of endogenous estrogens. It should be noted, however, that events contributing to changes in endogenous estrogen levels, also involve fluctuation in levels of other hormones such as progesterone, GnRH, FSH, LH, and oxytocin. Moreover, during the reproductive period and at later stages of life, women may be exposed to exogenous (i.e., from outside the body) estrogens through use of oral contraceptives (OC) or HT.

As aforementioned, evidence suggests that women have an increased risk of dementia, especially AD, compared to men.<sup>5,18,19,21,25,26</sup> Estrogen has been suggested as a possible explanation.<sup>3,28</sup> Whether estrogen acts neuroprotective, neurotoxic, or both, is still not fully understood despite decades of research.

#### 1.4.2.1 EXOGENOUS ESTROGENS AND DEMENTIA

Studies performed in animal or cellular models have reported neuroprotective effects of estrogen; estrogen has been shown to promote hippocampal health,<sup>130,131</sup> provide ischemic neuroprotection by improved brain perfusion,<sup>132</sup> enhance the functional status of cholinergic projections to the hippocampal formation and cortex,<sup>133</sup> and protect against the neurotoxicity from A $\beta$  aggregation.<sup>134</sup> Even though results from preclinical studies show clear beneficial effects of estrogen on neuronal health, results from studies including humans are not as conclusive. In observational studies, several studies report a reduced risk of dementia and AD in women taking exogenous estrogen,<sup>135-141</sup> while one study reported an increased risk of AD.<sup>142</sup> Further, one of the largest RCTs to investigate the relationship between exogenous estrogens and

dementia risk, the Women's Health Initiative Memory Study (WHIMS), reported a higher risk of dementia in postmenopausal women aged 65 years or older who were assigned estrogens plus progestin therapy compared to the placebo treatment group.<sup>143</sup> However, other RCTs have failed to find any association between exogenous estrogens and risk of dementia.<sup>144,145</sup>

#### 1.4.2.2 ENDOGENOUS ESTROGENS AND DEMENTIA

Similar to studies examining the effect of exogenous estrogen, results from studies examining the effect of endogenous estrogen on risk of cognitive decline and dementia are inconclusive. Studies examining levels of estradiol in serum have reported that higher<sup>146,147</sup> and lower<sup>148</sup> levels of estradiol (total or bioavailable) was associated with cognitive decline and dementia risk, while other studies did not find any association.<sup>149-151</sup>

Few longitudinal population-based studies have investigated the association between length of reproductive period, as an indicator of endogenous estrogen, and dementia risk. The Rotterdam Study found an association between longer reproductive period and increased risk of dementia and AD, especially in *APOE ε4* carriers.<sup>152</sup> In contrast, the Kaiser Permanente Study (KP) reported an increased risk of dementia in women with a shorter reproductive period.<sup>141,153</sup> In line with the KP study, a study based on the Korean National Health Insurance System database (NHIS) reported that a longer reproductive period was associated with reduced risk of dementia,<sup>141</sup> whereas the 10/66 study did not find any association.<sup>154</sup> The Rotterdam Study found that the effect of reproductive period on dementia and AD risk was mainly driven by age at menopause and not age at menarche,<sup>152</sup> while the KP study and the NHIS study reported that both later age at menarche and younger age at menopause were associated with increased risk of dementia.<sup>141,153</sup>

Moreover, several studies report that women having more children or more pregnancies have an increased risk of dementia.<sup>141,155-159</sup> In addition, breastfeeding has also been associated with dementia.<sup>141,160,161</sup>

#### 1.4.2.3 ESTROGENS AND BIOMARKERS FOR DEMENTIA

How about the effect of estrogens (exogenous or endogenous) on biomarkers for dementia and AD? Previous studies examining the association between biomarkers for dementia and estrogen, measured either as exogenous estrogen,<sup>162-164</sup> indicators of endogenous estrogen,<sup>165</sup> menopausal transition,<sup>164</sup> or levels of estradiol in serum,<sup>147</sup> have reported conflicting results.

The Kronos Early Estrogen Prevention Study (KEEPS) reported that women taking estrogen, compared to placebo, had less A $\beta$  deposition on Pittsburgh compound B-Positron emission tomography scans (PET), and less decline in dorsolateral prefrontal

cortex volumes on MRI.<sup>162</sup> In contrast, another report from the same study showed increased ventricular volumes and more WMH on MRI in women taking estrogen, compared to placebo.<sup>163</sup> Further, the Cardiovascular Health Study reported that women taking exogenous estrogens showed more prominent central atrophy on MRI, compared to women without estrogen treatment.<sup>166</sup>

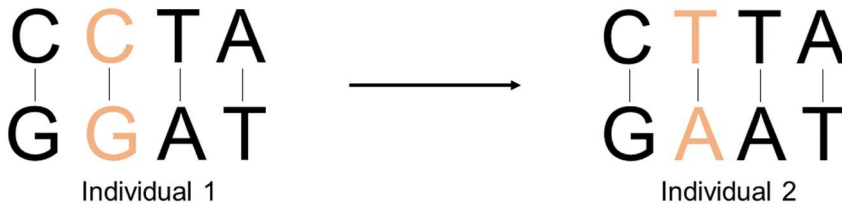
The UK biobank reported that women with longer reproductive period had reduced total brain volume (TBV), and that women with later menopausal age had reduced TBV and hippocampal volumes (HV) on MRI, compared to women with shorter reproductive period and earlier menopause.<sup>165</sup> The study also reported that women with later age at menarche had increased TBV than women with earlier.<sup>165</sup> In addition, the study showed that postmenopausal women had larger TBV and HV, compared to premenopausal women.<sup>165</sup> On the contrary, a three year follow-up study using MRI and PET in cognitively normal individuals reported that perimenopause- and menopausal women showed more indicators of AD, such as hypometabolism, increase A $\beta$  deposition, and reduced volumes of gray and white matter in AD-specific regions, compared to age- and education matched men and premenopausal women.<sup>164</sup>

Moreover, the Rotterdam Study reported that women with higher levels of estradiol in serum had smaller hippocampal volumes compared to women with lower levels, while no association was seen between estradiol levels and hippocampal volumes in men.<sup>147</sup> However, to date, no previous study has investigated the long-term effect of reproductive period on levels of CSF biomarkers for AD.

### 1.4.3 GENETIC FACTORS AND DEMENTIA

The DNA molecule is composed of a sugar-phosphate double strand carrying complementary guanine – cytosine and adenine – thymine base pairs, assembled into 23 chromosomes within the cells, comprising approximately  $3 \times 10^9$  base pairs and roughly 27,000 genes.<sup>167</sup>

The traditional manner to study monogenic diseases was through genetic linkage studies to detect the chromosomal location of the affected genes.<sup>168</sup> This approach relied on the knowledge that genes in close proximity on a chromosome remain linked during meiosis (i.e., cell division of germ cells).<sup>168</sup> In contrast to monogenic diseases, complex diseases, such as dementia, have a multifaceted genetic component caused by multiple genetic variants.<sup>78,169</sup> The most abundant form of genetic variation is single nucleotide polymorphisms (SNPs) that are single base-pair changes in the DNA sequence occurring in at least 1% of the population (Figure 5).<sup>167</sup>



**Figure 5.** Schematic illustration of a single nucleotide polymorphism (SNP). Source: Original by the author. The cytosine – guanine base pair in individual 1 is exchanged to base pair thymine – adenine in individual 2. Thus, the possible alleles for this SNP is C and T and as each gene has two copies an individual can have genotype CC, CT, or TT.

Mutations can also be caused by a single base-pair change.<sup>169</sup> However, in contrast to the high frequency of a SNP in the population, mutations are genetic variation with a low frequency.<sup>169</sup> Further, in contrast to the high effect (or penetrance) of genetic factors that cause monogenic disease, SNPs have relatively low effect on disease.<sup>169</sup> Therefore, to identify genetic variants associated with common diseases, studies with large sample sizes and large panels of genetic markers (i.e., genome-wide association studies [GWAS]) are needed.<sup>169</sup> Indeed, GWASs have helped identify SNPs associated with common diseases, such as LOAD.<sup>170-172</sup>

One important concept of GWAS is linkage disequilibrium (LD). LD refers to the linkage of two genetic markers on a population level and describe the degree of which an allele of a SNP is inherited with an allele of another SNP.<sup>169</sup> LD is generally reported in terms of  $R^2$  ( $R^2$  level 0 to 1), where high  $R^2$  values indicate that two SNPs are highly correlated.<sup>169</sup> In GWAS, LD is used to prevent genotyping of SNPs that contribute with redundant information.<sup>169</sup> Also, as GWAS analyses associations on large areas of the human genome, including millions of genetic markers, the risk of detecting false positive findings is high if the conventional  $P$  value threshold of  $<0.05$  is used. Therefore, based on the assumption of 1,000,000 independent associations, the significance level used in many GWASs is  $P$  value  $<5 \times 10^{-8}$  (i.e., genome-wide significance).<sup>173</sup>

#### 1.4.3.1 GENETIC FACTORS AND ALZHEIMER'S DISEASE

EOAD is almost completely genetically determined, with a heritability ranging from 92–100%.<sup>43,174</sup> Studies on autosomal dominant families with EOAD have discovered mutations in *APP* (chromosome 21), *PSEN1* (chromosome 14) and *PSEN2* (chromosome 1) genes, which mostly are inherited in a Mendelian fashion.<sup>46</sup> In total, 52 mutations have been identified in the *APP* gene, 215 in the *PSEN1* gene, and 31 in the *PSEN2* gene.<sup>43</sup> The frequency of these mutations among patients with EOAD is low ( $<1\%$  for *APP*,  $6\%$  for *PSEN1*, and  $1\%$  for *PSEN2*), and together they only explain 5–10% of EOAD patients.<sup>43</sup> Further, it should be noted that genes harboring rare

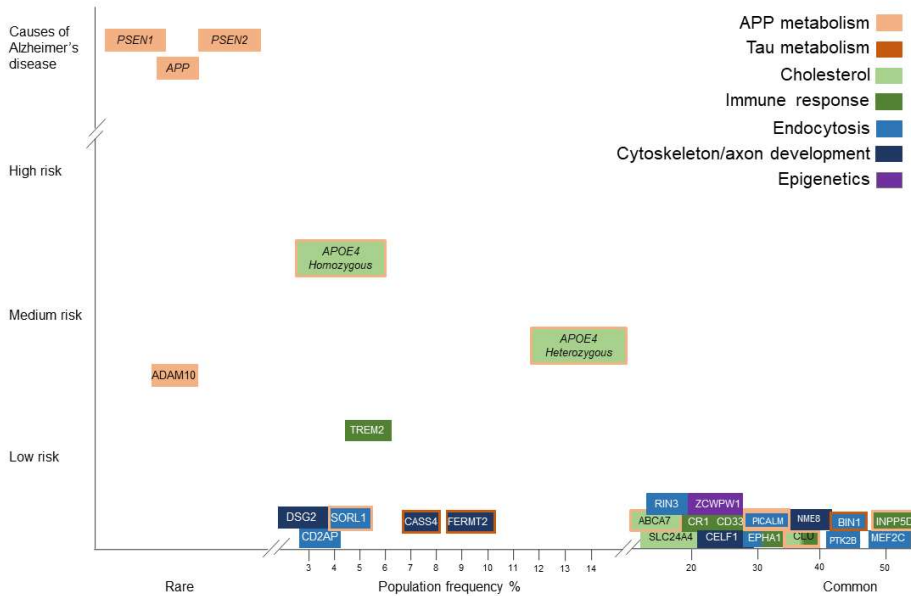


mutations involved in EOAD (e.g., *APP* and *PSEN1*), also include common genetic variations contributing to increased risk for LOAD.<sup>43</sup>

The etiology of LOAD is complex and the genetic component multifaceted.<sup>78</sup> The strongest genetic factor modulating the risk of LOAD is the *APOE* gene.<sup>79</sup> The protein comprises three isoforms, encoded by three different alleles; *APOE*  $\epsilon 2$  (protective allele), *APOE*  $\epsilon 3$  (neutral allele), and *APOE*  $\epsilon 4$  (risk allele). Apolipoprotein E (ApoE) is a lipid-binding glycoprotein. In the brain, ApoE transports cholesterol. Among the three isoforms, ApoE4 is the less efficient in reuse of membrane lipids and neuronal repair, and is also essential for A $\beta$  fibrillisation and plaque formation.<sup>44</sup> However, the pathological mechanisms for ApoE has not been fully established. Previous studies have reported an increased risk of AD by three times in individuals with one *APOE*  $\epsilon 4$  allele (heterozygotes) and by 15 times in those with two *APOE*  $\epsilon 4$  alleles (homozygotes), and a roughly 10 year earlier onset with each *APOE*  $\epsilon 4$  allele.<sup>44,175</sup> Also, in patients with EOAD, at least one *APOE*  $\epsilon 4$  allele increased the risk of AD, especially in those with a family history of disease.<sup>43</sup> Further, several studies have reported a pronounced effect of the *APOE*  $\epsilon 4$  allele among women than in men<sup>27</sup>; women with one  $\epsilon 4$  allele had four times higher risk of AD compared to  $\epsilon 4$  non-carriers, while men with one  $\epsilon 4$  allele showed less increased risk than women.

Moreover, in the oldest old (those aged 95 years and older), the effect of *APOE* genotype on dementia risk is still unclear; some studies report an effect of the *APOE*  $\epsilon 4$  allele on dementia risk,<sup>176 177</sup> while other studies have failed to find any association.<sup>178,179</sup>

GWASs have identified additional genetic variants that have shown genome-wide significant association with LOAD.<sup>170-172</sup> The genetic variants are involved in APP and Tau metabolism, inflammatory responses, cholesterol metabolism, endocytosis, cytoskeleton/axonal development, and epigenetics (Figure 6).<sup>57,168</sup> Compared to *APOE*  $\epsilon 4$  allele, the majority of these genetic variants have small effect on LOAD (odds ratio ranging from 0.8 to 1.2).<sup>172,180</sup> One exception is a very rare genetic variant of the *TREM2* gene (encoding a membrane protein that forms a complex with the TYRO protein tyrosine kinase-binding protein, activating the immune response)<sup>181</sup> that exhibits similar effect as the *APOE*  $\epsilon 4$  allele on LOAD (odds ratio approximately 2.0).<sup>180,181</sup> However, as the individual effect of most of these genetic variants on LOAD usually is small, they are often studied through the construction of polygenic risk scores (PRSs). PRSs include genetic variants that surpasses predefined *P* value thresholds using summary statistics from previous GWASs (e.g. Kunkle et al. and Lambert et al.).<sup>171,172</sup>



**Figure 6.** Schematic overview of genes associated with Alzheimer's disease (AD). Source: Figure by Scheltens et al. 2016,<sup>57</sup> with permission from Elsevier, adapted by the author. The y-axis denote the risk of developing AD for the genes. The x-axis denotes the frequency of the genes in the population. The colored boxes and the colored interiors explain the function of the genes. For example, APOE ε4 gene are involved in cholesterol metabolism as well as APP metabolism.

In PRSs, SNPs are extracted using LD clumping, extracting only one representative SNP per region of LD. After selecting SNPs, PRSs are calculated using effect sizes from a previous large GWAS multiplied with the number (or dosage) of the effect alleles of each genetic variant.

PRSs for AD (AD-PRSs) have been associated with cognitive decline in mixed samples (including individuals with MCI and AD),<sup>182,183</sup> in individuals with preclinical dementia<sup>184</sup> and MCI,<sup>185</sup> and in cognitively normal individuals.<sup>186,187</sup> Further, AD-PRSs have been associated with AD and dementia in clinical sample,<sup>188-192</sup> population-based samples,<sup>176,193-195</sup> and in pathological confirmed samples of AD.<sup>196</sup> Moreover, AD-PRSs have also been associated with pathological biomarkers for AD, such as cortical thickness,<sup>197</sup>  $\beta$ -amyloidosis and tau-pathology in the brain,<sup>59</sup> and CSF biomarkers (A $\beta$ 42, T-tau, P-tau, and neurofilament light chain [NfL]).<sup>185,198-200</sup> Thus far, studies examining the association between AD-PRS and risk of dementia in population-based samples including individuals aged 95 years above, are scarce.

#### 1.4.3.2 GENETIC FACTORS AND OTHER DEMENTIA SUBTYPES

Evidence from several GWASs show that genetic pleiotropy (i.e., one genetic variant is associated with different diseases) is a common feature of dementia.<sup>71,201</sup> One example of genetic pleiotropy is *APOE* genotype, which, in addition to AD, also has

been associated with VaD, FTD, and DLB.<sup>71</sup> In contrast to the more established genetic architecture of AD, the genetic architecture of other dementia subtypes has yet to be established. One important reason could be the heterogeneity of other dementia subtypes than AD.<sup>71</sup>

However, it should be noted that other dementia subtypes than EOAD also show a high family transmission and a Mendelian inheritance pattern. CADASIL (Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is the most common heritable cause of subcortical ischemic VaD, caused by mutations in the *NOTCH3* gene.<sup>202</sup> Another type of Mendelian VaD is CARASIL (cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy), an autosomal recessive disease (i.e., two copies of the mutation is needed to cause the disease) caused by mutations in the *HTRA1* gene.<sup>71,203</sup>

#### 1.4.3.3 GENETIC AND NON-GENETIC FACTORS IN RELATION TO DEMENTIA

Factors accumulating during the life course, intertwined in complex interactions, play a role in determining the individual risk of dementia. Several studies have tried to disentangle the complex interaction between genetic and non-genetic factors in relation to risk of dementia. However, the results are inconclusive; some studies report that the relation between lifestyle factors (e.g., physical activity, diet, alcohol consumption, and smoking) and risk of dementia and AD was modified by *APOE* genotype (a more pronounced effect in *APOE*  $\epsilon 4$  carriers<sup>115,204</sup> or  $\epsilon 4$  non-carriers<sup>193,205</sup>). In contrast, other studies have not found a modifying effect of *APOE* genotype.<sup>111,118</sup>

Further, the modifying effect of AD-PRS on the relation between non-genetic factors and dementia risk is also not fully understood. One study from the UK biobank, examining the interaction between an AD-PRS and a lifestyle score, reported that genetic risk and lifestyle factors were independently associated with risk of dementia.<sup>194</sup>

## 2 RATIONALE

Given the expected increase in people with dementia, it is important to study risk factors for dementia to identify individuals at increased risk in order to implement preventative strategies before dementia pathology start to accumulate. Despite decades of research, there are still knowledge gaps to fill.

First, studies examining the effect of marital status on risk of other comorbidities than dementia, such as depression,<sup>103,104</sup> hypertension,<sup>105</sup> and death,<sup>105</sup> show that unmarried men have a higher risk of disease and death compared to married men. Several studies have reported an association between marital status and risk of dementia and AD.<sup>84,87-95</sup> However, studies examining the modifying effect of sex on this association are scarce. Therefore, in *Paper I*, we examined the modifying effect of sex on the relation between marital status and risk of dementia, in two longitudinal population-based samples from Rochester, Minnesota (MN), USA, and Gothenburg, Sweden.

Second, regardless of the numerous studies examining the effect of cognitive and physical activity on dementia risk, the studies report inconsistent results.<sup>83,106-110</sup> Possible explanations for the inconsistencies could be that many studies have investigated the relationship between the activities and incident dementia in samples of individuals at higher ages at baseline, when information on activity levels was obtained, and in studies with shorter observations periods. To minimize the possibility that the association may be affected by preclinical dementia, long-term studies examining the association between midlife cognitive and physical activity and risk of dementia are needed. In addition, few studies have examined the independent effect of physical and cognitive activity on the risk of dementia. We therefore studied the independent role of midlife cognitive and physical activity on risk of dementia and dementia subtypes in a population-based sample of women followed over 44 years in *Paper II*.<sup>206</sup>

Third, given the differences in the lifetime risk of dementia between men and women, it is also important to study sex differences in risk factors for dementia. Estrogen has been suggested as a possible explanation to the increased dementia and AD risk in women compared to men.<sup>3,28</sup> Although several studies have examined the association between exogenous estrogen and dementia risk,<sup>135-140,142-145</sup> few previous studies have examined the long-term association between reproductive period and other indicators of endogenous estrogens and dementia risk.<sup>141,152-154</sup> Therefore, in *Paper III*, we examined the long-term association between indicators of endogenous estrogens, measured as reproductive period, age at menarche and menopause, number of pregnancies, and months of breastfeeding, and risk of dementia and dementia subtypes in women followed over 44 years.<sup>207</sup> In addition, few studies have examined the

association between estrogens and biomarkers for dementia and AD and as far as we know no previous study has examined the association between reproductive period, as an indicator of endogenous estrogen, and levels of CSF biomarkers for AD. Therefore, in *Paper IV*, we examined the long-term association between reproductive period and levels of CSF biomarkers for AD (A $\beta$ 42, P-tau, T-tau, and ratio A $\beta$ 42/A $\beta$ 40) in a population-based sample of women free from dementia and with a natural menopause, followed over 25 years.

Fourth, the influence of the complex genetic component of LOAD on dementia risk in the general population needs to be further investigated, especially in the oldest old (those age 95 years or older). Therefore, in *Paper V*, we examined if AD-PRSs, the *APOE* genotype, and the interaction of these, were associated with risk of dementia in a population-based sample of individuals aged 70–111 years.<sup>208</sup>

### 3 AIM

The overarching aim of this thesis was to study risk and protective factors in relation to dementia and dementia subtypes, with focus on lifestyle-, particularly marital status and leisure time cognitive and physical activity, hormonal-, particularly indicators of endogenous estrogen, and genetic factors. The thesis contains five papers based on population-based samples from the Gothenburg H70 Birth Cohort studies (the H70-studies), the Prospective Population Study of Women (PPSW), and the Mayo Clinic Study of Aging (MCSA 70+ study).

The specific aims of all papers:

**Paper I.** To explore the modifying effect of sex on the relation between marital status and risk of dementia, in two longitudinal population-based samples from Rochester, Minnesota (MN), USA and Gothenburg, Sweden.

**Paper II.** To explore the independent role of midlife cognitive and physical activity on risk of late-life dementia and dementia subtypes in a population-based sample of women followed over 44 years.<sup>206</sup>

**Paper III.** To explore the longitudinal association between indicators of endogenous estrogen, measured as reproductive period, age at menarche and menopause, number of pregnancies, and months of breastfeeding, and risk of dementia and dementia subtypes among women with natural menopause who were followed over 44 years.<sup>207</sup>

**Paper IV.** To explore the long-term association between reproductive period, as an indicator of endogenous estrogen, and levels of CSF biomarkers for AD (A $\beta$ 42, P-tau, T-tau, and ratio A $\beta$ 42/A $\beta$ 40) in a population-based sample of women free from dementia and with natural menopause, followed over 25 years.

**Paper V.** To explore the effects of AD-PRSs, the *APOE* genotype, and the interaction of these, on the risk of dementia in a large population-based study of individuals aged 70–111 years.<sup>208</sup>

*“If we knew what it was we were doing, it would not be called research,  
would it?”*

Albert Einstein

## 4 MATERIALS AND METHODS

### 4.1 STUDY POPULATIONS

The population-based samples used in this thesis were derived from the Gothenburg H70 Birth Cohort Studies (the H70-studies; including the H70, H75, H85, H88, H90, and 95+ studies), the Prospective Population Study of Women (PPSW), and the Mayo Clinic Study of Aging (MCSA 70+ study), from Rochester (MN), USA.

The H70-studies are multidisciplinary longitudinal studies examining representative birth cohorts of men and women aged 70 years or older in Gothenburg, Sweden.<sup>209-211</sup> The study was initiated in 1971–72 with the examination of those born 1901–02, as previously described (Figure 7).<sup>210</sup> Briefly, in 1971–72 a systematically selected sample of individuals aged 70 years was obtained from the Swedish Population Registry.<sup>210</sup> Individuals registered in Gothenburg and born between the 1<sup>st</sup> of July, 1901 and the 30<sup>th</sup> of June, 1902, on dates ending with 2, 3, 5 or 8, were sampled.<sup>210</sup> In total, 1007 individuals participated in the complete examination or with telephone interviews only (participation rate 87.7%).<sup>210</sup> This birth cohort, along with additional birth cohorts, have been followed longitudinally with almost identical study protocols (Figure 7).<sup>209-211</sup> In 2014–16, the most recent and largest birth cohort of 70-year-olds, born 1944, were examined (n=1,203) (Figure 7).<sup>209</sup> Further, the 95+ study started in 1996–98 including individuals aged 95 years and born between 1<sup>st</sup> of July, 1901 and 31<sup>st</sup> of December, 1903 (Figure 7).<sup>212</sup> In total, 338 individuals were examined (participation rate 65%).<sup>212</sup> Examinations were performed at ages 95, 97, and 99 years and every year thereafter.<sup>212</sup> Since then, several birth cohorts aged 95 years or older have been examined (Figure 7).

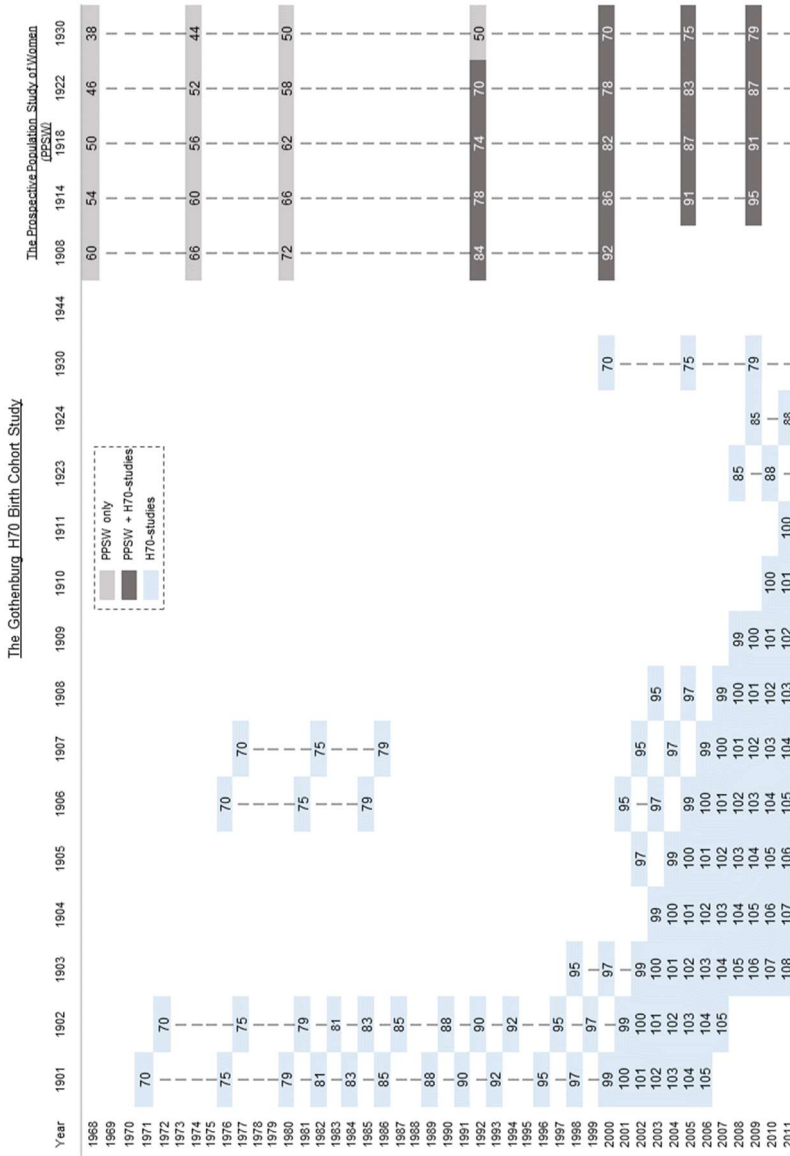
PPSW is a multidisciplinary longitudinal study, examining representative samples of women living in Gothenburg, Sweden. In 1968–69, a systematically selected sample of 1622 women born 1908, 1914, 1918, 1922, and 1930 was invited to a health examination. The women were selected from the Swedish Population Registry based on specific birth dates to yield a representative sample (Table 1).<sup>213</sup>

**Table 1.** Date of participant selection and ages at baseline for PPSW in 1968–69.

Birth cohort	Date of participant selection	Mean age (SD)
1908	6	60.9 (0.2)
1914	6, 12	54.6 (0.2)
1918	6, 12, 18, 24, 30	50.6 (0.2)
1922	6, 12, 18, 24, 30 <sup>a</sup>	46.6 (0.2)
1930	6, 12, 18, 24, 30 <sup>a</sup>	38.6 (0.2)

Source: Bengtsson et al. 1973.<sup>213</sup> <sup>a</sup>Only those born in January until June on the 30<sup>th</sup> were invited.





**Figure 7.** Overview of the examinations of birth cohorts included in *Paper I–V* from the Gothenburg H70 Birth Cohort studies (the H70-studies) and the Prospective Population Study of Women (PPSW). Source: Original scheme created by Thomas Marlow, adapted by the author. X-axis depicts the study of origin and birth year of all cohorts. Y-axis depicts year of examination. Numbers in cells depicts age at examination. Cells colored in light blue depicts the examinations of participants included in the H70-studies (H70 study, H75 study, H85 study, H88 study, H90 study, and the 95+ study). Cells colored in light grey display the examinations of women included in only PPSW, while cells colored in dark grey indicate the examination where PPSW and the H70-studies were merged.

In total, 1462 women participated (participation rate 90%) in 1968–69.<sup>213</sup> All women were invited to re-examinations in 1974–75 (n=1,302), 1980–81 (n=1,154), 1992–93 (n=836), 2000–02 (n=662), 2005–07 (n=536), 2009–11 (n=351), and 2015–16 (n=180), with participation rates of 91%, 84%, 72%, 74%, 74%, 73%, and 73%, respectively. In addition to the women examined in 1968–69, additional women were examined in 1980–81 (n=47) and 1992–93 (n=34) to ensure representative samples at the ages studied.<sup>214,215</sup> In 1992, women aged 70 years or older (i.e., those born 1922, 1918, 1914, and 1908) were also examined with the H70-study battery (Figure 7). Further, in 2000–02, the H70-studies and PPSW (women born 1908, 1914, 1918, 1922, and 1930) were merged (Figure 7).

The MCSA 70+ study is a population-based longitudinal study, described in more detail previously.<sup>216,217</sup> Briefly, Olmsted County (MN, USA) residents aged 70–89 years on October 1, 2004, were enumerated using the Rochester Epidemiology Project (REP) medical records-linkage system (n=20,805 individuals had been in contact with the system at least once within three years prior to the index date).<sup>216,218</sup> After excluding duplicated records for the same persons (n=8,752) and non-residents (n=2,100), 9,953 participants were enumerated.<sup>216</sup> In total, 5,233 individuals were considered for eligibility, of which 4,398 individuals were included in the eligible sample (263 died prior to contact, 56 were terminally ill or in hospice, 114 could not be contacted to confirm eligibility, and 402 had dementia and were therefore not contacted).<sup>216</sup> Participants were re-examined every 15-months using the same clinical protocol for evaluation as in 2004.

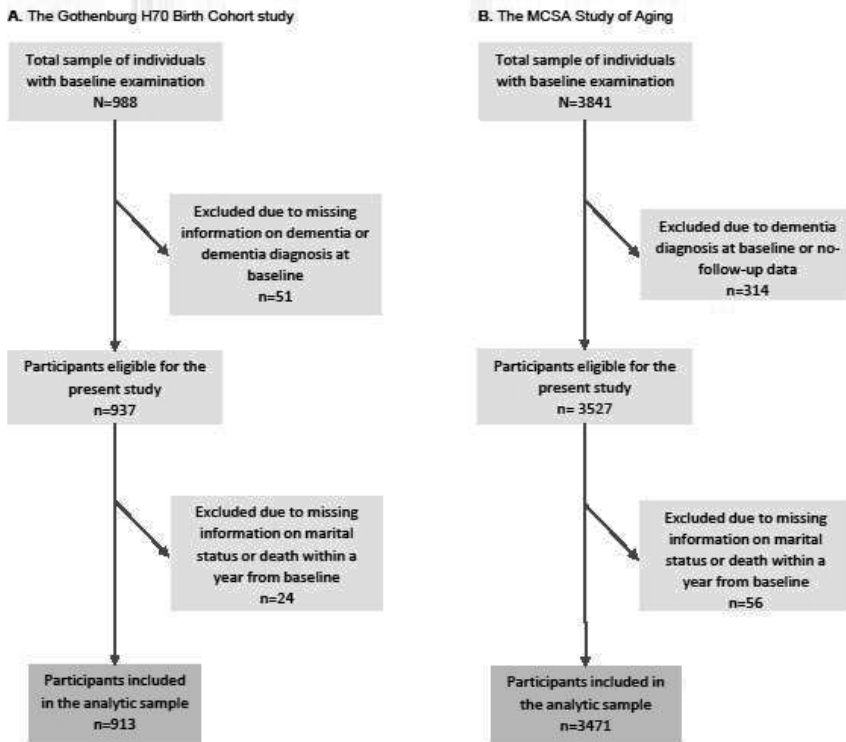
#### 4.1.1 STUDY POPULATION OF PAPER I

In *Paper I*, participants were a part of the H70-studies, examined in 2000–02 and 2005–07, and re-examined in 2009–11, and 2015–16,<sup>219,220</sup> and from the MCSA 70+ study, examined in 2004 and re-examined every 15-months.<sup>212,213</sup>

In the H70-studies, 982 participants born 1930 had a baseline examination and information on dementia status. After exclusion of 45 participants with dementia at baseline, 937 individuals were considered eligible for *Paper I* (Figure 8). In total, 913 of 937 participants from the H70-studies were included in the analytic sample (22 participants were excluded due to missing information on marital status, and two due to death within a year from baseline) (Figure 8).

In the MCSA 70+ study, 3,891 participants aged 70–89 years had a baseline examination, of which 50 women were excluded due to living in a convent. After exclusion of 120 participants with dementia at baseline and 194 participants due to no follow-up data, 3,527 participants were considered eligible for *Paper I* (Figure 8). In total, 3,471 of 3,527 participants from the MCSA 70+ study were included in the

analytic sample (seven participants were excluded due to missing information on marital status and 49 due to death within a year from baseline) (Figure 8).



**Figure 8.** Study flow chart of participants included in *Paper I*, from (A) the Gothenburg H70 Birth Cohort Studies (H70-studies), and (B) the Mayo Clinic Study of Aging (MCSA 70+ study), from Rochester, MN, USA. Source: Based on the method section and Figure 1 from *Paper I*, modified by the author.

#### 4.1.2 STUDY POPULATION OF PAPER II

A subsample of the women invited to participate in PPSW in 1968–69 were systematically selected for a psychiatric examination ( $n=899$ ).<sup>221</sup> The women were born 1914, 1918, 1922, and 1930 and selected based on specific dates.<sup>221</sup> In total, 800 women participated in the psychiatric examination (participation rate 89%).<sup>206,221</sup> The women were invited to re-examinations in 1974–75 ( $n=677$ ), 1980–81 ( $n=625$ ), 1992–93 ( $n=371$ ), 2000–02 ( $n=371$ ), 2005–07 ( $n=300$ ), and 2009–11 ( $n=182$ ), with participation rates of 86%, 83%, 68%, 75%, 77%, and 76%, respectively.

After excluding those with missing information on cognitive and physical activity, 784 women were included in the analytic sample of *Paper II* (Figure 9).

### 4.1.3 STUDY POPULATION OF PAPER III

In total, 1543 women born 1908, 1914, 1918, 1922, and 1930 were examined at least one time in 1968–69, 1974–75, 1980–81, or 1992–93 (Figure 9). After excluding 59 participants due to missing information on indicators of endogenous estrogen (age at menarche or menopause, number of pregnancies, or months of breastfeeding), 92 due to premature (defined as menopause before age 38 years) or surgical menopause (i.e., hysterectomy and/or uni- or bilateral oophorectomy prior to menopause), and 28 due to missing information on dementia, 1,364 women with natural menopause were included in the analytic sample of *Paper III* (Figure 9).<sup>207</sup>

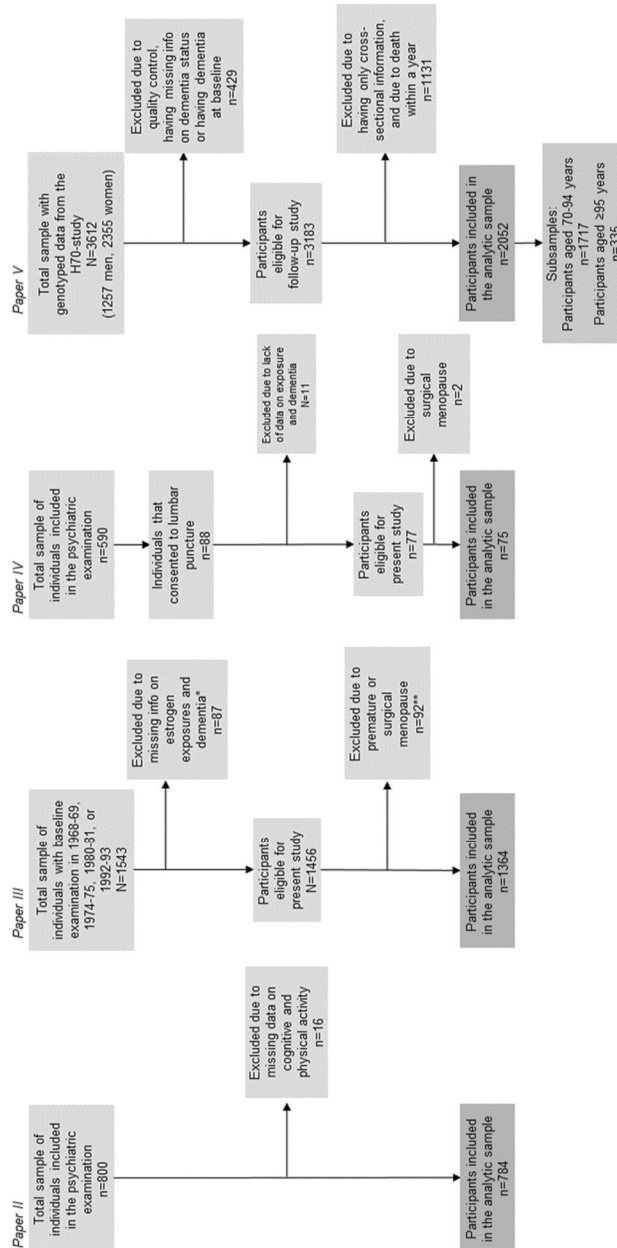
### 4.1.4 STUDY POPULATION OF PAPER IV

In the follow-up examination 1992–93, a subsample of women born 1908, 1914, 1918, and 1922 participated in an extensive psychiatric examination with the H70-study battery (n=590, participation rate 68%), of which 88 consented to a lumbar puncture (LP).<sup>222</sup> After excluding seven women due to lack of information on reproductive period, four due to dementia, and two due to surgical menopause, 75 cognitively normal women with natural menopause were included in the analytic sample of *Paper IV* (Figure 9).

### 4.1.5 STUDY POPULATION OF PAPER V

We used a sample of 3,612 participants born 1901–11, 1914, 1918, 1922–24, 1930, and 1944, with genotyped data from the H70-studies (Figure 7 and Figure 9). The participants were examined at least once between 2000 and 2016.<sup>208-211</sup>

In total, 3,449 of 3,612 had genotyped data after performing quality control (QC) and information on dementia status.<sup>208</sup> After excluding 266 individuals with dementia at baseline, 3,183 participants were eligible for *Paper V*. Of these, 1,118 were excluded due to having cross-sectional information only and 13 due to death within a year from baseline, leaving 2,052 individuals for the analytic sample (Figure 9).<sup>208</sup> The total analytic sample was further divided into two subsamples based on age at blood sampling (70–94 years and 95 years or older). In those aged 70–94 years (born 1914, 1918, 1922–24, and 1930), 1,717 were followed in relation to incident dementia. Among those aged 95 years or older (born 1901–11), 335 were followed in relation to incident dementia (Figure 9).<sup>208</sup>



**Figure 9.** Study sample flow charts of *Paper II–V*. Source: Based on the method section and study flow charts from *Paper II–V*,<sup>206-208</sup> adapted by the author. *Paper III*: \*28 had missing data on dementia status, and one woman had dementia prior to menopause. \*\*27 women were excluded due to premature menopause (six at age 21–29 and 22 at age 31–37). In total, 64 women were excluded due to surgical menopause (i.e., with hysterectomy and/or unilateral or bilateral oophorectomy prior to menopause).

## 4.2 THE GENERAL HEALTH EXAMINATION OF THE H70-STUDIES AND PPSW

The examinations were similar for the H70-studies and PPSW, and has been practically identical at every examination for both studies. As aforementioned, women aged 70 years and older from the PPSW were included in the H70-study battery in 1992–93, and since the examination in 2000–02 the two studies are merged. The examinations in PPSW prior to the merge in 2000–02 has been described in more detail previously.<sup>213-215,223</sup> The participants were examined at an out-patient clinic or at the residence of the participant for those who had difficulties to come to the clinic. Experienced research nurses, psychologists, or medical doctors performed the examinations.

The participants' went through a comprehensive health examination, including semi-structured interviews, physical examinations, and psychometric testing for the measure of cognitive health. The interviews included questions regarding present and past medical status, social and sociodemographic factors (e.g., education, occupation, and income), reproductive history, past and present psychiatric health, activities of daily living (ADL) and instrumental activities of daily living (IADL), and lifestyle factors (e.g., cigarette smoking, alcohol consumption, marital status, and cognitive and physical activity). The physical examinations included electrocardiogram (ECG), anthropometric measurements (e.g., height and length), blood pressure, and blood sampling (e.g., measures of total cholesterol, LDL, HDL, triglycerides, and glucose). In addition to the general health examination, participants were asked to participate in computed tomography (CT), magnetic resonance imaging (MRI), lumbar puncture (LP), dietary examination, audiological and ophthalmological examinations, and the participants' were asked to name a close informant to participate in a close informant interview.

### 4.2.1 NEUROPSYCHIATRIC EXAMINATION AND PSYCHOMETRIC TESTING

The neuropsychiatric interview included questions on history of psychiatric illness/disorder, suicidal behavior, sleeping patterns, and cognitive symptoms related to dementia disorders. Psychiatric symptoms and signs common in dementia were rated according to the Comprehensive Psychopathological Rating Scale (CPRS),<sup>224</sup> Gottfries-Bråne-Steen Scale (GBS),<sup>225</sup> and the Clinical Dementia Rating (CDR).<sup>226</sup>

Psychometric tests examining memory, orientation, apraxia, visuospatial function, understanding proverbs, following commands, naming ability and language, were performed using the Mini-Mental State Examination (MMSE)<sup>227</sup> and assessments similar to the Alzheimer's Disease Assessment Scale (ADAS-COG).<sup>228-230</sup> Tests

included from ADAS-COG were word-recall task (naming 12 items that were shown to the participant, then recalling the items after approximately two and five minutes), naming fingers and objects, following commands, copying/drawing figures (a cross, circle, rhombus, two rectangles, and a cube), ideational practice (five steps to send a letter to one self), orientation, word recognition (10 words are shown to the participant), ratings of language abilities (spoken abilities, word finding abilities, and comprehension of spoken language), ratings of concentration, depressive mood, tearfulness, delusions, hallucinations, pacing, and increased motor activity.<sup>209,228</sup>

Inter-rater reliability between psychiatrists and nurses was examined in 50 participants. The Kappa values ranged from 0.74 to 1.00 for the presence versus absence of symptoms and signs that were used to diagnose dementia (e.g., memory, language, visuospatial ability, and apraxia).<sup>229</sup> In addition, it should be emphasized that Professor Ingmar Skoog, who has been the principal investigator of the H70-studies since the 80s, trained all personnel performing the neuropsychiatric examinations.

## 4.2.2 ADDITIONAL EXAMINATIONS

### 4.2.2.1 CLOSE INFORMANT INTERVIEWS

In the H70-study battery, psychiatric research nurses or psychologists performed semi-structured close informant interviews over the phone. The interviews comprised questions about changes in intellectual function, behavior and personality, psychiatric symptoms, and performance in ADL and IADL, and in case of dementia, age of onset and disease course.<sup>230</sup>

### 4.2.2.2 CEREBROSPINAL FLUID SAMPLING

Lumbar punctures (LPs) were conducted between 1992 and 1993, for women born 1908, 1914, 1918, and 1922. LPs were performed through the L3/L4 interspace and CSF samples of 12 mL were collected and gently mixed to avoid gradient effects.<sup>231</sup> To eliminate cells and other insoluble materials, the samples were centrifuged at 2,000 g for 10 min and stored at  $-80^{\circ}\text{C}$  in 1-mL polypropylene vials until analyses.<sup>232,233</sup> Sandwich enzyme-linked immunosorbent assays (ELISAs) were used to determine levels of A $\beta$ 42, A $\beta$ 40, P-tau, and T-tau.<sup>232-234</sup> In *Paper IV*, we used CSF levels of A $\beta$ 42, P-tau, T-tau, and ratio of A $\beta$ 42 and A $\beta$ 40.

## 4.2.3 MEDICAL RECORDS AND REGISTRY DATA

For women included in PPSW, medical records were collected from all inpatient and outpatient departments and general practitioners' offices in Gothenburg. For PPSW and the H70-studies, the National In-Patient Register provided diagnostic information for all participants discharged from hospitals on nationwide basis from 1978–2012. In

addition, information on deaths during follow-up was obtained from the Swedish population register for all participants until December 31, 2016.

#### 4.2.4 DEMENTIA DIAGNOSIS AND DEMENTIA SUBTYPES

In the H70-study battery, dementia was diagnosed according to criteria similar to DSM-III-R in three steps based on information from neuropsychiatric examination, psychometric testing, and close informant interviews: 1) dementia was diagnosed according to computerized algorithms, 2) the algorithm-based dementia diagnoses were reviewed by at least two psychiatrists, and 3) a final dementia status was determined at a consensus conference that included at least two psychiatrists.<sup>30,230</sup> Evaluators were blinded to information and diagnoses from previous examinations. First, individuals who had impairment in short- or long-term memory, and with symptoms of either constructional difficulties, impaired abstract thinking, impaired judgement, aphasia, agnosia, apraxia, or personality changes were assigned a dementia diagnosis according to a computerized algorithm based on information from the neuropsychiatric examinations and the psychometric tests. Also, individuals who had impairment in short- or long-term memory and with symptoms of either impaired orientation, aphasia, difficulties with ADL, reduced interests and initiative, or personality changes were assigned a dementia diagnosis according to a computerized algorithm based on information from the close informant interviews. Each symptom had to have attained a level at which it caused the subject substantial difficulty in social functioning.<sup>230</sup> Second, at least two psychiatrists reviewed the computerized algorithms from the neuropsychiatric examinations and the close informant interviews individually and confirmed or refused the diagnosis set by the algorithms. Third, the cases that the psychiatrists were in disagreement about were discussed in a consensus conference in order to determine a final dementia status.

Dementia diagnosis for women examined in PPSW before 1992–93 and for individuals lost to follow-up, were based on information from medical records evaluated by psychiatrists in consensus conference, and the Swedish Inpatient Register.<sup>230</sup>

Individuals with dementia were classified into dementia subtypes according to the cause of the dementia. Probable or possible AD was diagnosed according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association (NINCDS-ADRDA) criteria (Appendix 3).<sup>40</sup> VaD was diagnosed with criteria similar to those outlined by the National Institute of Neurological Disorders and Stroke–Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) criteria (Appendix 4).<sup>235</sup> VaD was diagnosed when there was a temporal relationship (within 1 year) between a history of stroke or transient ischemic attack (TIA) and the first signs



of neurocognitive impairment. The diagnosis of stroke and/or TIA was based on self- or proxy-reported history of focal neurological symptoms (e.g., hemiparesis or aphasia) and self- or proxy-reported duration of symptoms (threshold of 24 h was used to differentiate stroke from TIA), and the Swedish Inpatient Register, as described previously.<sup>236</sup> Mixed dementia was diagnosed when both AD and CVD were judged to contribute to dementia.<sup>206</sup> Dementia with CVD included individuals with dementia and stroke/TIA without consideration of the temporal relationship between dementia and stroke/TIA.<sup>207</sup> Based on information from close informant interviews and register data, FTD, PDD, alcohol dementia, dementia due to normal pressure hydrocephalus, Huntington's disease, brain tumor, and brain trauma, and unspecified dementia were diagnosed.

Information provided by close informants, the Swedish Inpatient Register, and the examinations was used to determine age at dementia onset. If no information could be obtained from these sources, the mid-point between last examination free from dementia and the first with a dementia diagnosis was used to determine age at dementia onset.<sup>206,207</sup>

## 4.2.5 POTENTIAL CONFOUNDERS

Information on potential confounders was obtained either through semi-structured interviews (education, socioeconomic status [SES], cigarette smoking, angina pectoris, and psychological stress) or through health examinations (hypertension, waist-hip-ratio [WHR], body mass index [BMI], diabetes mellitus, and ECG, and dyslipidemia) or by a combination of these (hypertension, diabetes mellitus, and myocardial infarction). In *Paper I* and *V*, years of education was used, while educational attainment was dichotomized as compulsory (6 years for those born 1908–1922 and 7 years for those born 1930), or more in *Paper II–III*. In *Paper IV*, educational attainment was categorized into four groups. Group 1 had  $\leq 6$  years of education, group 2 had 7–9 years of education, group 3 had 10–12 years of education, and group 4 had  $>12$  years of education. SES was based on husband's occupation for married women and own occupation for unmarried women and was defined as high (upper middle class and above), medium (lower middle class), and low (working class).<sup>206,237</sup> Cigarette smoking was defined as number of cigarettes per day in *Paper II*, and dichotomized as current/former smoker and non-smoker in *Paper I, III, and IV*. WHR was defined as the ratio between waist and hip circumference. BMI was calculated as  $\text{kg/m}^2$ . In *Paper II–IV*, hypertension was defined as taking antihypertensive medication, systolic blood pressure  $\geq 160$  mm Hg, or diastolic blood pressure  $\geq 95$  mm Hg. In *Paper I*, hypertension was defined as taking antihypertensive medication, systolic blood pressure  $\geq 140$  mm Hg, or diastolic blood pressure  $\geq 95$  mm Hg. In *Paper I, III and IV*, diabetes mellitus was defined as a diagnosis told by a doctor, being on antidiabetic treatment, or having one venous blood glucose value of  $\geq 11.1$  mmol/L. In *Paper IV*,

diabetes mellitus was defined as a diagnosis told by a doctor, being on antidiabetic treatment, or having two venous blood glucose values of  $\geq 7.0$  mmol/L. Dyslipidemia was defined as having a ratio of total cholesterol and high density lipoprotein (HDL)  $\geq 5$  mmol/l, low density lipoprotein (LDL)  $\geq 3.5$ , or taking lipid-lowering drugs. Angina pectoris was diagnosed according to Rose criteria.<sup>238</sup> The diagnosis of ischemic heart disease was defined as having angina pectoris, myocardial infarction, and/or ECG changes. ECG was performed on all participants during rest, and ischemic heart disease was diagnosed according to Minnesota codes 1.1–2, 4.1, 5.1–2 (in absence of 3.1), 6.1, or 7.1.<sup>239</sup> Women who reported frequent or constant stress symptoms, such as tension, nervousness, and sleeping disturbance ( $\geq 1$  month) in relation to circumstances in everyday life, were considered to have psychological stress. In *Paper II*, major depressive episode was diagnosed according to DSM-III-R,<sup>30</sup> using information from the psychiatric interview. In *Paper I*, using information from the psychiatric interview, a composite variable of any depression was used, including those with a diagnosis of minor depression (according to DSM-IV-TR)<sup>42</sup> or major depression (according to DSM-5).<sup>32,240</sup>

#### 4.2.6 MARITAL STATUS

Information on current marital status was obtained in 2000–02 and 2005–07. Participants reported if they were married, cohabiting with a partner-not married, single-never married, divorced, widowed, or in a relationship but living apart (“*särbos*” in Swedish). In *Paper I*, to examine the difference in dementia risk between those who were married/in a marriage-like relationship and those who were not, marital status was dichotomized as “married” (married and cohabitant with a partner) and “not married” (single-never married, divorced, widowed, and in a relationship but living apart).

#### 4.2.7 COGNITIVE ACTIVITY

In 1968–69, five leisure time cognitive activities were assessed: intellectual, artistic, manual, club, and religious. The frequency of each activity was rated as none/low (score 0), moderate (score 1), or high (score 2). None/low was determined when the woman did not participate in the different activities.<sup>206</sup> Examples of the moderate and high degrees are shown in Table 2.

**Table 2.** Cognitive activities in a moderate or high degree.

Cognitive activity	Degree of leisure time cognitive activity	
	Moderate	High
Intellectual	Read a book last 6 months	More reading, writing
Artistic	Visit a concert, theatre, art exhibition last six months	Frequent visits, plays on instrument, sings in a choir, paints pictures
Manual	Needlework last 6 months, gardening last year	Several interests
Club	Membership	Board member
Religious	Church attendance at least few times last year	Church attendance at least 12 times last year

The cognitive activities were assembled to a sum score based on the frequency level (score 0–2). For example, a woman who had none/low participation in intellectual activities (0 points), moderate degree of participation in artistic activities (1 point) and manual activities (1 point), and high degree of participation in religious activities (2 points) had a sum score of 4 points. Further, a woman who had none/low participation in all cognitive activities had a sum score of 0 points, while a woman who had a high degree of participation on all cognitive activities had a sum score of 10 points.<sup>206</sup> In *Paper II*, cognitive activity was dichotomized as 0–2 (inactive) vs 3–10 (active) based on the median of engagement.<sup>206</sup>

## 4.2.8 PHYSICAL ACTIVITY

The Saltin-Grimby Physical Activity Level Scale (SGPALS) was used to interview participants regarding physical activity. SGPALS has shown predictive validity in relation to cardiovascular risk factors and mortality.<sup>241-243</sup> On the basis of this measure, each woman was assigned to one of four groups.<sup>206</sup> Group 1 was completely inactive, e.g., at most looking at television and going to the movies. Group 2 engaged in light physical activity at the minimum of four hours per week, such as walking, gardening, bowling or cycling for half an hour a day. Group 3 had regular physical training, such as running, tennis, or swimming, for at least two–three hours per week. Group 4 had regular to intense physical training such as heavy exercise e.g., running or swimming several times per week, or engaging in competitive sports.<sup>206</sup> In *Paper II–IV*, physical activity was dichotomized as inactive (group 1) and active (group 2–4) based on the distribution of engagements.<sup>206</sup>

## 4.2.9 INDICATORS OF ENDOGENOUS ESTROGENS

Information on reproductive history (i.e., age at menarche and menopause, type of menopause, number of pregnancies and miscarriages, and months of breastfeeding) was obtained in 1968–69, 1974–75, 1980–81, and 1992–93, covering the entire reproductive period of all birth cohorts from PPSW (those born 1908, 1914, 1918, 1922, and 1930; n=1,543).<sup>207</sup> Age at menarche was defined as first menstruation, and the first given information was used.<sup>207</sup> Menopause was defined as one year without menstruation.<sup>207</sup> The first given information on menopausal age was used to obtain

information on age at menopause close to the actual event.<sup>207</sup> Reproductive period was defined as time from age at menarche to age at menopause.<sup>207</sup> In *Paper III* and *Paper IV*, age at menarche, menopause, and reproductive period was used as continuous variables.<sup>207</sup> In *Paper III*, reproductive period was also divided into quartiles.<sup>207</sup>

Number of pregnancies since the last examination was reported in 1974–75 and 1980–81.<sup>207</sup> In 1968–69, the women reported number of children and miscarriages. Number of pregnancies in 1968–69 was defined as the sum of children and miscarriages.<sup>207</sup> Information on months of breastfeeding was reported in 1968–69, 1974–75, 1980–81, and 1992–93.<sup>207</sup> The last given information on numbers of pregnancies and months of breastfeeding was used to ensure coverage of all events.<sup>207</sup> In *Paper III* and *Paper IV*, number of pregnancies and months of breastfeeding was used as continuous variables. In *Paper IV*, number of miscarriages was dichotomized as women who reported no miscarriages and women who reported one or more miscarriages.

#### 4.2.10 EXOGENOUS ESTROGEN

Information on use of OC was reported in 1968–69, 1974–75, 1980–81, and 1992–93 and HT in 1992–93.<sup>207</sup> In *Paper III*, use of OC and HT were merged into exogenous estrogens (former users of OC or HT vs non-users), as the duration of use for both HT and OC was skewed.<sup>207</sup> In *Paper IV*, OC and HT were used separately. To deal with the high proportion of women who never used OC or HT, both a continuous variable and a dichotomous variable (previous user vs never user) of OC and HT were used.<sup>244</sup>

#### 4.2.11 GENETIC ANALYSES

In 2000–11 and 2014–16, blood sampling for genetic analyses was performed.<sup>208</sup> Genotyping was performed with the NeuroChip (Illumina).<sup>245</sup> QC included the exclusion of individuals with the following: per-sample call rate <98%, sex mismatch, and excessive heterozygosity (FHET [F coefficient estimate for assessing heterozygosity] outside  $\pm 0.2$ ).<sup>208</sup> Further, samples were identified as non-European ancestral outliers and excluded if their first two principal components (PCs) exceeded six standard deviations from the mean values of the European samples in the 1000 Genome global reference population.<sup>208</sup> Closely related samples were removed based on pairwise PI\_HAT (i.e., proportion of genome that are in identity-by-descent; calculated using --genome option in PLINK)  $\geq 0.2$  (i.e., first and second degree relatives were excluded).<sup>208</sup> Also, markers were removed due to per-SNP call rate <98%, minor allele frequency (MAF) <0.01, and Hardy-Weinberg disequilibrium ( $P < 1 \times 10^{-6}$ ).<sup>208</sup>

The Sanger imputation service was used to impute post-QC, using the reference panel of Haplotype Reference Consortium data (HRC1.1).<sup>208</sup> Post-imputation QC included removal of SNPs with low imputation quality (RSQ [imputation  $R^2$ ]  $\leq 0.3$ ).<sup>208</sup> The

mean RSQ for the SNPs included in the AD-PRSs (described in more detail below) was 0.83 (SD 0.13).<sup>208</sup>

The variants rs7412 and rs429358 (which define the  $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$  alleles) in the *APOE* gene were also genotyped with the KASPar® PCR SNP genotyping system (LGC Genomics, Hoddesdon, Herts, UK) or by mini-sequencing, as previously described in detail.<sup>208,246</sup>

## 4.2.12 POLYGENIC RISK SCORES

AD-PRSs were constructed using summary statistics from stage 1 of the most recent AD GWAS including clinically defined AD.<sup>172,208</sup> SNPs were selected using LD-clumping. Briefly, the European ancestry samples from the 1000-genomes project were used as reference panel to remove variants in LD, all variants 250kb upstream and downstream of top signal were removed ( $R^2 < 0.001$ ).<sup>208</sup> All variants in the *APOE* region (chromosome 19, coordinates hg19 [GRCh37]: 44412079 to 46412079) were removed.<sup>208</sup> We created PRS including variants that surpassed four *P* value thresholds ( $P < 1e^{-5}$ ,  $P < 1e^{-3}$ ,  $P < 1e^{-1}$ ), referred to as  $1e^{-5}$  AD-PRS (including 57 SNPs),  $1e^{-3}$  AD-PRS (including 1,333 SNPs), and  $1e^{-1}$  AD-PRS (including 13,942 SNPs).<sup>208</sup> For the  $p < 5e^{-8}$  level, we used an AD-PRS based on 39 SNPs (39-SNPs AD-PRS)<sup>208</sup> that have shown genome-wide significant association with AD after combined meta-analyses in the most recent GWAS by de Rojas et al.<sup>170</sup> In the same study, the 39-SNPs AD-PRS was also validated for the first time in a clinical sample.<sup>170</sup>

All AD-PRSs were calculated as the sum of the  $\beta$ -coefficient multiplied with the number (or dosage) of effect alleles of each SNP (information on SNPs included in the  $1e^{-5}$  AD-PRS and the 39-SNPs AD-PRS is shown in Supplementary Table 1 for *Paper I*).<sup>208</sup>

The population was further divided into low-, middle-, and high-risk tertiles of AD-PRSs.<sup>208</sup> To avoid boundaries being affected by survival to old age, limits of the tertiles were calculated using data from 1,130 participants born 1944 (mean age at blood sampling 70.6 years, SD 0.3 years).<sup>208</sup> The AD-PRSs were standardized and used as continuous variables in all analyses, while tertiles of AD-PRSs were used to stratify the data.<sup>208</sup>

## 4.2.13 *APOE* GENOTYPE

In *Paper III* and *Paper V*, *APOE* genotype was divided into  $\epsilon 4$  carriers ( $\epsilon 4/\epsilon 2$ ,  $\epsilon 4/\epsilon 3$ , or  $\epsilon 4/\epsilon 4$ ) and  $\epsilon 4$  non-carriers ( $\epsilon 2/\epsilon 2$ ,  $\epsilon 3/\epsilon 3$ , or  $\epsilon 3/\epsilon 2$ ). In *Paper V*, *APOE* genotype was also divided into  $\epsilon 2$  carriers ( $\epsilon 2/\epsilon 2$ ,  $\epsilon 2/\epsilon 3$ ,  $\epsilon 2/\epsilon 4$ ) and  $\epsilon 2$  non-carriers ( $\epsilon 3/\epsilon 3$ ,  $\epsilon 3/\epsilon 4$ ,  $\epsilon 4/\epsilon 4$ ).

## 4.3 THE MCSA 70+ STUDY EXAMINATION

The examinations were performed at the Mayo Clinic Abigail Van Buren Alzheimer's Disease Research Clinic, Rochester (MN), USA, or at the place of residence of the participant.<sup>216</sup> The examinations included an evaluation and risk factor assessment performed by a nurse or a study coordinator, a neurological interview performed by a physician with a specialty in neurology, geriatrics, or psychiatry, and a neuropsychological evaluation performed by a psychometrist. The evaluation and risk factor assessment, performed by a nurse or a study coordinator, included blood sampling, anthropometric measurements, assessment of family history, current medication, demographic information, memory and orientation, the PRIME MD (participant form) for evaluation of mental disorders,<sup>247</sup> medical history and risk assessment, neuropsychiatric inventory, the CDR,<sup>248</sup> and the Functional Activities Questionnaire (FAQ).<sup>249</sup> The neurological interview included a neurological interview, the Short test of Mental Status,<sup>250</sup> modified Hachinski Scale<sup>251,252</sup>, PRIME MD (physician form), neurological examination, and modified Unified Parkinson's Disease Rating Scale.<sup>216</sup> The neuropsychological evaluation included subsets of the Wechsler Adult Intelligence Scale Revised (WAIS-R), the Wechsler Memory Scale-Revised (WMS-R), and other psychometric tests to assess four cognitive domains (memory [Logical memory II - delayed recall, Visual Reproduction II – delayed recall, and Auditory Verbal Learning Test – delayed recall]<sup>253</sup>, executive functions [Trail Making Test B<sup>254</sup> and Digit symbol Substitution], visuospatial functions [Picture completion and Block Design], and language [Boston Naming Test<sup>255</sup> and Category Fluency<sup>256</sup>]).<sup>216</sup>

### 4.3.1 DEMENTIA DIAGNOSIS

Diagnosis of dementia was based on DSM-IV,<sup>30</sup> using information from the evaluation by a nurse or study coordinator, the neurological interview, and the neuropsychological evaluation. For each visit, all information from the evaluation was reviewed by the nurse or study coordinator, the physician, and the neuropsychologist and a dementia diagnosis or normal cognition was adjudicated by consensus.<sup>216,217</sup> Evaluators were blinded to information and diagnoses from previous examinations. Age at onset was determined as age at the examination where dementia was first diagnosed.

### 4.3.2 POTENTIAL CONFOUNDERS

Information on potential confounders used in *Paper I* was based on information from the study examination (education, number of children, BMI, and smoking status) or nurse abstracted from the participants medical records using the REP medical record-linkage system described on page 26 (hypertension, depression, dyslipidemia, and diabetes mellitus). Years of education and number of biological children were used as

continuous variables. BMI was defined as  $\text{kg}/\text{m}^2$ . Smoking status was dichotomized as ever and never smoker.

### 4.3.3 MARITAL STATUS

Information on current marital status was obtained through self-report at baseline. Participants were asked if they were married, living together-not married, single-never married, divorced, widowed, or separated. In *Paper I*, to examine the difference in dementia risk between those who were married/in a marriage-like relationship and those who were not, marital status was dichotomized as “married” (married and cohabitant with a partner) and “not married” (single-never married, divorced, widowed, and separated).

## 4.4 STATISTICAL ANALYSES OF PAPER I

### Summary box for *Paper I*

**Aim:** To explore the modifying effect of sex on the relation between marital status and risk of dementia, in two longitudinal population-based samples from Rochester, Minnesota (MN), USA and Gothenburg, Sweden.

**Exposures:** Marital status ("married" [including those married and cohabiting with a partner] vs "not married" [including those single never-married, divorced, widowed, and separated in the MCSA 70+ study and those in a relationship but living apart in H70-studies])

**Outcome:** Incident all-cause dementia.

**Total sample from MCSA 70+ study:** n=3,471.

**Total sample for the H70-studies:** n=913.

**Statistical method:** Cox regression models using age as time-scale, presented as Hazard ratios (HR) and 95% confidence interval (CI).  $P < 0.05$  was considered statistically significant.

**Time at risk:** Participants were censored at the date of a) dementia diagnosis, b) death, or c) end of follow-up. End of follow-up for the MCSA 70+ study cohort was defined as September 12, 2019. End of follow-up for the H70-studies cohort was defined as December 31, 2016 for those with last examination year in 2015–16, and December 31, 2012 for those with last examination year in 2009–11 and register data until 2012. The proportional hazard assumption was met for all Cox regression models.

**Model 1:** Marital status, baseline age, and sex.

**Model 2:** Marital status, baseline age, sex, years of education, and number of children.

**Model 3:** Depression, BMI, hypertension, dyslipidemia, and diabetes mellitus in addition to covariates included in model 2.

#### **Analyses\*:**

- 1) The association between marital status and risk of all-cause dementia in model 1–3.
- 2) Interaction of sex and marital status in relation to risk of all-cause dementia using model 3.
- 3) Re-analyses were performed in samples separated by sex in model 1–3.
- 4) Marital status in relation to all-cause mortality in a Cox regression model adjusted for baseline age, sex, years of education, number of children, any depression, BMI, hypertension, dyslipidemia, and diabetes mellitus. Also, analyses examining the interaction between sex and marital status in relation to risk of mortality in fully adjusted models. Finally, marital status in relation to all-cause mortality in fully adjusted Cox regression models separated by sex.

All analyses were performed with R (version 3.6.1) using stats, ggplot2, survival, and survminer packages.

**Figure 10.** Summary of boxes for *Paper I*. Source: based on the method section from *Paper I*, summarized and adapted by the author. \*All analyses were performed in the two population-based samples separately.



## 4.5 STATISTICAL ANALYSES OF PAPER II

### Summary box for *Paper II*

**Aim:** To explore the independent role of midlife cognitive and physical activity on risk of late-life dementia and dementia subtypes in a population-based sample of women followed over 44 years.

**Exposures:** Midlife cognitive and physical activity.

**Outcomes:** Incident all-cause dementia, AD, VaD, mixed dementia, and dementia with CVD.

**Total sample:** n=784.

**Statistical method:** Cox regression models using risk-time as time scale, presented HR and 95% CI.  $P < 0.05$  was considered statistically significant.

**Time at risk:** Time at risk was calculated from baseline examination in 1968–69 until a) year of dementia onset; b) date of death for those who died during follow-up; or c) December 31, 2012 for those with last examination year in 2009–11 and register data until 2012. The proportional hazard assumption was met for all Cox regression models.

**Model 1:** Age and cognitive and physical activity separately.

**Model 2:** Age and both activities simultaneously.

**Model 3\*:** Age, both activities simultaneously and relevant covariates:

**Analyses:**

- 1) The association between cognitive and physical activity and risk of all-cause dementia, AD, VaD, mixed dementia, and dementia with CVD in model 1–3.
- 2) Re-analyses were performed after categorizing cognitive activity into quartiles and physical activity into tertiles, to investigate a potential dose-response relationship.
- 3) To minimize the risk of preclinical dementia affecting the results, re-analyses were performed after excluding those who developed dementia before 1990 (i.e., 22 years after baseline; n=21).
- 4) For the purpose of this thesis, we examined the association between midlife cognitive and physical activity and risk of mortality, in a Cox regression model adjusted for education, SES, BMI, hypertension, cigarettes per day, diabetes, angina pectoris, psychological stress, and major depression.

All analyses were performed with R (version 3.6.1) using stats, ggplot2, survival, and survminer packages or IBM SPSS STATISTICS for Windows v.23 (IBM Corp., Armonk, NY, USA).

**Figure 11.** Summary box for *Paper II*. Source: based on the method section from Najar et al. 2019,<sup>206</sup> summarized and adapted by the author. \*Covariates for model 3 were selected in a primary analysis, where each potential confounder (education, SES, hypertension, smoking, diabetes mellitus, angina pectoris, psychological stress, and major depression) was analyzed in relation to dementia disorders using age, cognitive and physical activity as covariates in the model. Covariates related to dementia disorders at  $P$  value threshold  $< 0.3$  were included.<sup>206</sup>

## 4.6 STATISTICAL ANALYSES OF PAPER III

### Summary box for *Paper III*

**Aim:** To explore the longitudinal association between indicators of endogenous estrogen, measured as reproductive period, age at menarche and menopause, number of pregnancies, and months of breastfeeding, and risk of dementia and dementia subtypes among women with natural menopause who were followed over 44 years.

**Exposures:** Age at menarche and menopause, reproductive period, number of pregnancies, and months of breastfeeding.

**Outcomes:** Incident all-cause dementia, AD, and dementia with CVD.

**Total sample:** n=1,364.

**Statistical method:** Cox regression models using risk-time as time scale, presented as HR and 95% CI.  $P < 0.05$  was considered statistically significant.

**Time at risk:** Time at risk was calculated from the examination year when menopausal age was first reported until a) year of dementia onset; b) date of death for those who died during follow-up; or c) December 31, 2012 for those with last examination year in 2009–11 and register data until 2012. The proportional hazard assumption was met in all Cox regression models.

**Model 1:** Each indicator of endogenous estrogen and birth year.

**Model 2:** Indicators of endogenous estrogen, birth year, and exogenous estrogens.

**Model 3\*:** In addition to the covariates included in model 2, relevant covariates were added to model 3.

**Analyses:**

- 1) The association between indicators of endogenous estrogens and risk of all-cause dementia, AD, and dementia with CVD in model 1–3.
- 2) Re-analyses were performed in samples stratified by four age groups (<65 years, 65–74 years, 75–84 years, ≥85 years).
- 3) To minimize the risk of preclinical dementia affecting the results, re-analyses were performed excluding those who developed dementia before 2000 (n=144).
- 4) Subsample of 603 women with information on *APOE* genotype: Interaction between *APOE*  $\epsilon 4$  carriership and length of reproductive period and age at menopause, respectively, in relation to incident dementia and AD (using model 1–3).
- 5) The association between reproductive period and age at menopause and risk of all-cause mortality in a Cox regression model adjusted for birth year, exogenous estrogens, physical activity, WHR, smoking status, hypertension, psychological stress, diabetes mellitus, ischemic heart disease, and dementia incidence.

All analyses were performed with R (version 3.6.1) using stats, ggplot2, survival, and survminer packages or IBM SPSS STATISTICS for Windows v.23 (IBM Corp., Armonk, NY, USA).

**Figure 12.** Summary box for *Paper III*. Source: based on the method section in Najar et al. 2020,<sup>207</sup> summarized and adapted by the author. \*Covariates for model 3 were selected in a primary analysis, where each potential confounder (education, physical activity, smoking, angina pectoris, stress, hypertension, WHR, diabetes mellitus, and myocardial infarction) was analyzed in relation to dementia disorders, using birth year and each endogenous estrogen exposure as covariates in the model. Covariates related to dementia disorders at  $P$  value threshold <0.3 were included.<sup>207</sup>

## 4.7 STATISTICAL ANALYSES OF PAPER IV

### Summary box for *Paper IV*

**Aim:** To explore the long-term association between reproductive period, as an indicator of endogenous estrogen, and levels of CSF biomarkers for AD (A $\beta$ 42, P-tau, T-tau, and ratio A $\beta$ 42/A $\beta$ 40) in a population-based sample of women free from dementia and with natural menopause, followed over 25 years.

**Exposures:** Reproductive period

**Outcomes:** A $\beta$ 42, P-tau\*, T-tau\*, and ratio A $\beta$ 42/A $\beta$ 40

**Total sample:** n=75.

**Statistical method:** Linear regression models, presented as  $\beta$ -coefficients, standard errors (SE), and R<sup>2</sup>.

**Model 1:** Reproductive period and birth year.

**Model 2\*\*:** In addition to the variables included in model 1, relevant covariates were included in model 2.

**Analyses:**

- 1) The association between reproductive period and levels of CSF biomarkers for AD, in model 1–2.
- 2) Re-analyses were performed after excluding major outliers (i.e., 3×IQR) from P-tau (n=1).
- 3) In separate analyses, we examined the two components of reproductive period, age at menarche and menopause, in relation to CSF biomarkers for AD in a crude model including either age at menarche or menopause and birth year.

All analyses were done with R (version 3.6.1) using stats and ggplot2 packages.

**Figure 13.** Summary box for *Paper IV*. Source: based on the method section from *Paper IV*, summarized and adapted by the author. \*P-tau and T-tau were natural log transformed to improve symmetry of the distributions. \*\*Covariates for model 2 were selected in a primary analysis, where each potential confounder (education, number of pregnancies, months of breastfeeding, OC, HT, angina pectoris, stress, physical activity, smoking, hypertension, WHR, ECG, and myocardial infarction) was analyzed in relation to CSF biomarkers for AD, using birth year and reproductive period as covariates in the model. Covariates related to the different CSF biomarkers, at *P* value threshold <0.3, were included.

## 4.8 STATISTICAL ANALYSES OF PAPER V

### Summary box for *Paper V*

**Aim:** To explore the effects of AD-PRSs, the *APOE* genotype, and the interaction of these, on the risk of dementia, in a large population-based study of individuals aged 70–111 years.

**Exposures:** AD-PRSs and *APOE* genotype.

**Outcome:** Incident all-cause dementia.

**Total sample:** n=2,052.

**Participants aged 70-94 years:** n=1,717.

**Participants aged ≥95 years:** n=335.

**Statistical method:** Cox regression models using age as time scale, presented as HR and 95% CI.  $P < 0.05$  was considered statistically significant.

**Covariates:** Age at blood sampling, birth year, sex, and 10 principal components to correct for population stratification.

**Time at risk:** Participants were censored at the date of a) dementia diagnosis, b) death, or c) end of follow-up (December 31, 2016 for those with last examination year in 2015–16, and December 31, 2012 for those with last examination year in 2009–11 and register data until 2012). The proportional hazard assumption was met for all Cox regression models.

**Analyses:**

- 1) The association between AD-PRSs and *APOE* genotype and risk of all-cause dementia.
- 2) Interaction analyses between *APOE* genotype (based on  $\epsilon 4$  or  $\epsilon 2$  carriership in separate models) and AD-PRSs in relation to risk of all-cause dementia.
- 3) Based on the results from the interaction analyses: The association between AD-PRSs and risk of all-cause dementia stratified by  $\epsilon 4$  carriership, and the association between  $\epsilon 4$  carriership and risk of all-cause dementia stratified by AD-PRSs (39-SNPs AD-PRS and  $1e^{-5}$  AD-PRS, respectively).
- 4) Re-analyses were performed in a subsample excluding  $\epsilon 4/\epsilon 2$  heterozygotes (i.e.,  $\epsilon 4$  carriers comprised  $\epsilon 4/\epsilon 3$  heterozygotes and  $\epsilon 4/\epsilon 4$  homozygotes, and  $\epsilon 2$  carriers comprised  $\epsilon 3/\epsilon 2$  homozygotes and  $\epsilon 2/\epsilon 2$  homozygotes).
- 5) Re-analyses were performed in a subsample of individuals with genotyped data and information on MMSE and years of education (n=1,394), to adjust for MMSE score at baseline and years of education.
- 6) The association between AD-PRSs (the 39-SNPs AD-PRS and the  $1e^{-5}$  AD-PRS) and *APOE* genotype and risk of all-cause mortality in a Cox regression model using the same covariates as listed above. We also examined the interaction between *APOE*  $\epsilon 4$  carriership and the two AD-PRSs, separately, in relation to all-cause mortality.
- 7) The individual effect of the different SNPs included in the AD-PRSs (the 39-SNPs AD-PRS and the  $1e^{-5}$  AD-PRS) on risk of all-cause dementia in a Cox regression model adjusted for the same covariates as listed above. In addition, analyses excluding the SNPs associated with incident dementia, one by one, to examine if any of those SNPs drove the associations with the AD-PRSs.

All analyses were done with R (version 3.6.1) using stats, ggplot2, survival, and survminer packages.

**Figure 14.** Summary box for *Paper V*. Source: based on the method section from *Paper V*,<sup>208</sup> summarized and adapted by the author.

## 4.9 ETHICAL CONSIDERATIONS

Since the examination in 1976 for the H70-studies and 1980 for PPSW, the Regional Ethical Review board in Gothenburg approved all studies including samples from the H70-studies and the PPSW and all participants gave informed consent prior participation according to the Helsinki declaration. If the participant was unable to provide own consent, consent was obtained from a next of kin. Information regarding potential risks, expected duration of the examination, information regarding storage and handling of personal data, register data approval, and the freedom to interrupt the examination at any time was given in the invitations letter before the examination.

The examinations could be regarded as extensive and demanding for the participants, including several tests and interviews. Still, most participants have chosen to partake in re-examinations over several decades. Also, for those who had difficulties to come to the clinic, home visits were offered. Furthermore, a medical doctor (“legitimerad läkare”) reviewed the results from the psychiatric and somatic health examinations. If pathologies or undiagnosed disease were detected (e.g., high blood pressure, psychiatric disorders, atrial fibrillation, diabetes mellitus), participants were referred to an appropriate health clinic for further examination and treatment.

In *Paper I*, for the MCSA 70+ study, the Institutional Review Boards of the Mayo Clinic and of the Olmsted Medical Center approved all study procedures and ethical aspects. All participants were informed of the scope of the project and signed an informed consent form including a Health Insurance Portability and Accountability Act (HIPAA) authorization.<sup>216</sup>

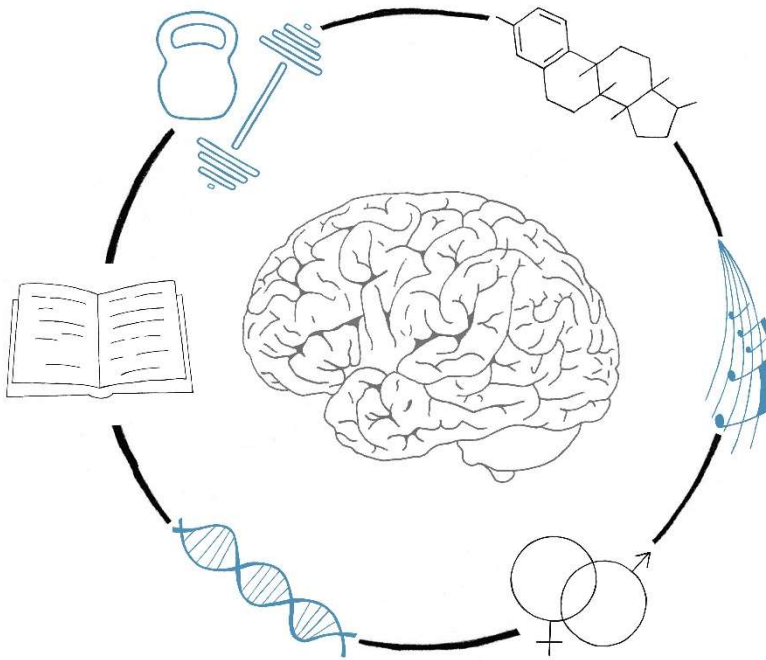
Registration (DNR) and reference numbers for all examinations included in this thesis are found in Appendix 5.

*“I was taught that the way of progress was neither swift nor easy.”*

Marie Curie

## 5 RESULTS

Results for all papers included in this thesis are summarized below. A more detailed description can be found in the separate articles and manuscripts at the end of this thesis.



**Figure 15.** The figure illustrates the association between brain health and factors examined in this thesis (lifestyle, hormonal, and genetic factors). Source: Original by Jenna Najjar, illustrated by Elliot Pettersson.

## 5.1 RESULTS OF PAPER I

### Summary results for the H70-studies (*Paper I*).

**Mean (SD) follow-up time:** 10 years (4 years)  
**Mean (SD) age at baseline:** 73 years (3 years)  
**Person-years of follow-up:** 9,470  
**N of dementia cases:** 149  
**Mean (SD) age at dementia onset:** 80 years (4 years)  
**N censored due to death:** 224  
**Median age at death (min, max):** 80 years (71, 86 years)  
**Marital status:** 63% were married at baseline

### Summary results for the MCSA 70+ study (*Paper I*).

**Mean (SD) follow-up time:** 7 years (4 years)  
**Mean (SD) age at baseline:** 79 years (5 years)  
**Person-years of follow-up:** 23,608  
**N of dementia cases:** 631  
**Mean (SD) age at dementia onset:** 87 years (6 years)  
**N censored due to death:** 459  
**Median age at death (min, max):** 88 years (73, 102 years)  
**Marital status:** 67% were married at baseline

As this paper has not been published, this section provides a very short summary of the results. A more detailed description of the results can be found in *Paper I*.

In summary, in both studies, married men had a reduced risk of all-cause dementia compared to unmarried men, while no association was observed between marital status and risk of all-cause dementia in women.

Further, in both studies, being married was associated with reduced risk of all-cause mortality compared to those unmarried. There was no significant interaction between sex and marital status in relation to risk of mortality. Nevertheless, analyses separated by sex showed that married men had a reduced risk of mortality compared to unmarried men, while no association was observed in women.



## 5.2 RESULTS OF PAPER II

**Summary results for Paper II, based on results published in Najar et al. Cognitive and physical activity and dementia: A 44-year longitudinal population study of women. Neurology. 2019.**

**Mean (SD) follow-up time:** 44 years (10 years)

**Mean (SD) age at baseline:** 53 years (6 years)

**Person-years of follow-up:** 26,322

**N of all-cause dementia cases:** 194\*

**N of AD cases:** 102

**N of VaD cases:** 27

**N of mixed dementia cases:** 41

**N of dementia with CVD cases:** 81\*\*

**Mean (SD) age at dementia onset:** 80 years (8 years)

**N censored due to death:** 596

**Mean (SD) age at death:** 80 years (10 years)

**The sum score of cognitive activities:** Peak at sum score 2 (21%) and 3 (19%)

**Physical activity:** 70% were physically activity on a regular basis (group 2)

\*AD + VaD + mixed dementia + other dementias

\*\*including some VaD cases and mixed dementia cases

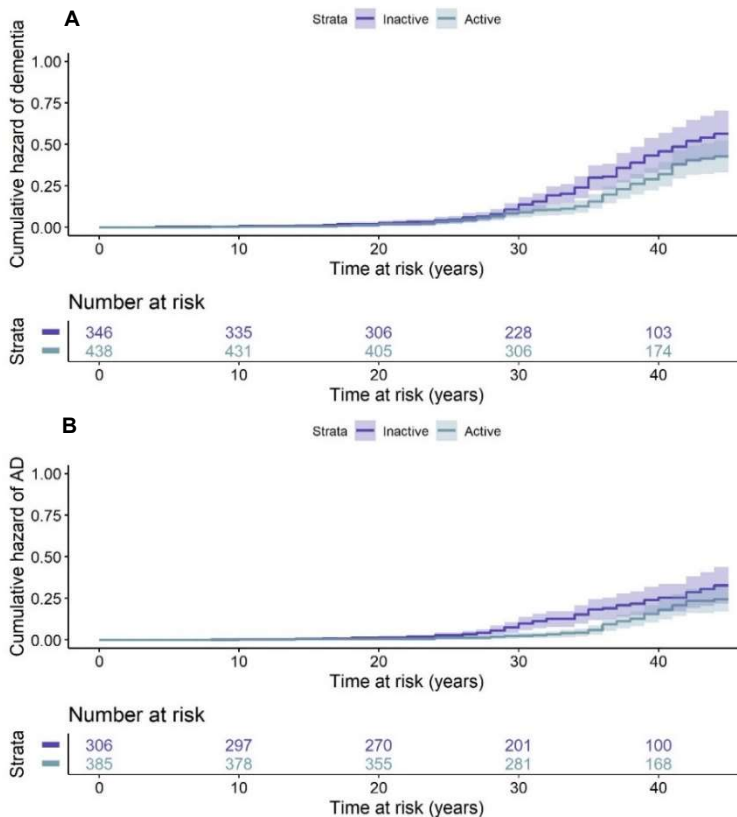
**Table 3.** Sociodemographic factors and health characteristics by physical and cognitive activity.

Characteristics	Physical activity		Cognitive activity	
	Inactive (Group 1)	Active (Group 2–4)	Inactive (Level 0–2)	Active (Level 3–10)
Age (years), mean (SD)	48 (4)	47 (5)	47 (5)	47 (5)
Education (more than compulsory), % (cases/total number)	29 (40/137)	29 (189/655)	14 (47/348)	41 (180/436)
Socioeconomic status (high), % (cases/total number)	59 (81/138)	61 (399/656)	48 (169/351)	71 (311/439)
Smoking (cig. per day), mean (SD)	6.0 (8)	4 (6)	5 (7)	4 (6)
Stress (score 3–5), % (cases/total number)	23 (32/138)	18 (115/656)	17 (59/348)	20 (86/438)
BMI, mean (SD)	25 (4)	24 (4)	25 (4)	24 (4)
Systolic blood pressure (mm Hg), mean (SD)	134 (24)	133 (21)	134 (22)	133 (21)
Diastolic blood pressure (mm Hg), mean (SD)	87 (11)	85 (11)	86 (11)	85 (11)
Hypertension, % (cases/total number)	28 (39/138)	19 (126/656)	22 (77/348)	20 (87/438)
Diabetes, % (cases/total number)	2 (3/138)	0 (1/656)	1 (4/348)	0 (0/438)
Angina Pectoris, % (cases/total number)	1 (1/138)	1 (3/656)	1 (3/348)	0 (1/438)
Major depression, % (cases/total number)	10 (14/138)	7 (46/656)	9 (31/351)	7 (29/439)

Source: Based on Table 1 in Najar et al. 2019,<sup>206</sup> with permission from Wolters Kluwer health, Inc., adapted by the author.

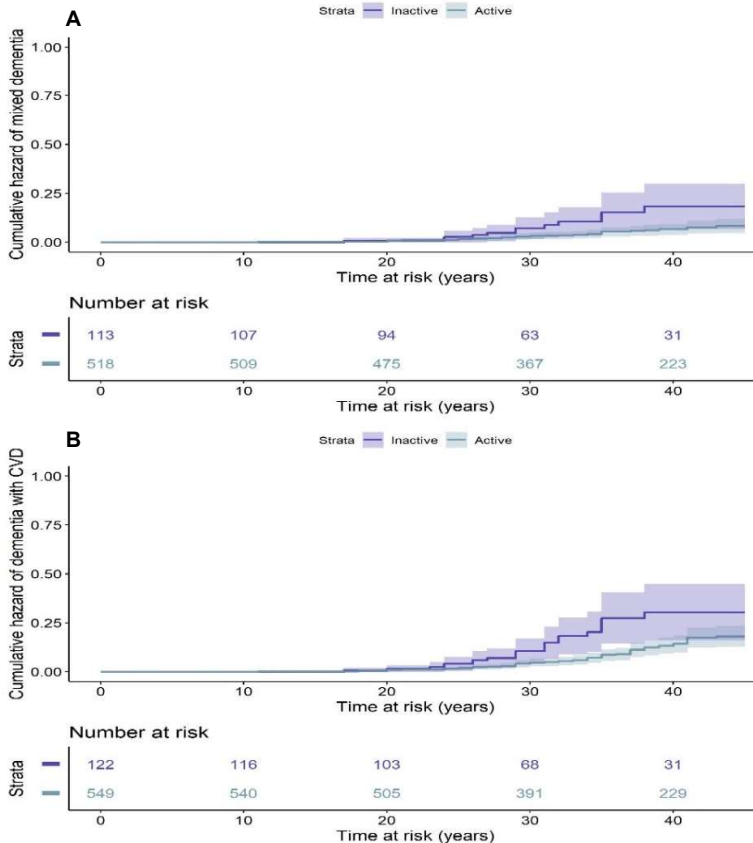
Sociodemographic factors and sample characteristics of *Paper II* are shown in Table 3. Compared to those cognitively inactive (level 0–2), cognitively active (level 3–10) women had higher educational attainment ( $P<.0001$ ), higher SES ( $P<.0001$ ), lower BMI ( $P<.0001$ ), and smoked less cigarettes ( $P=.02$ ). Compared to those physically inactive (Group 1), physically active (Group 2–4) women had lower proportion of hypertension ( $P=.02$ ) and smoked less cigarettes ( $P=.002$ ).

Midlife cognitive activity was associated with reduced risk of all-cause dementia (HR 0.66; 95% CI 0.49–0.89) and AD (HR 0.54; 95% CI 0.36–0.82) in fully adjusted models (model 3, Figure 16).<sup>206</sup>



**Figure 16.** Cumulative hazard of (A) all-cause dementia and (B) Alzheimer’s disease (AD), by cognitive activity (inactive [level 0–2] vs active [level 3–10]). The y-axis depicts the cumulative hazard, and the x-axis depicts time at risk shown in years. Analyzes adjusted for age and physical activity set to sample average. Levels of cognitive activity are demonstrated in different colors, with shaded areas showing the 95% confidence intervals. Source: Original by the author, based on the results published in Najjar et al. 2019.<sup>206</sup>

Midlife physical activity was associated with reduced risk of mixed dementia (HR 0.43; 95% CI 0.22–0.86) and dementia with CVD (HR 0.47, 95% CI 0.28–0.78) in fully adjusted models (model 3, Figure 17).<sup>206</sup>



**Figure 17.** Cumulative hazard of (A) mixed dementia and (B) dementia with cerebrovascular disease (CVD), by physical activity (inactive [level 0–1] vs active [level 2–4]). The y-axis depicts the cumulative hazard, and the x-axis depicts time at risk shown in years. Analyses adjusted for age and cognitive activity set to sample average. Levels of physical activity are demonstrated in different colors, with shaded areas showing the 95% confidence intervals. Source: Original by the author, based on the results published in Najjar et al. 2019.<sup>206</sup>

Further, the results did not change after exclusion of those with dementia before 1990 (n=21), with the exception that midlife physical activity was associated with all-cause dementia in these analyses (HR 0.67; 95% CI 0.46–0.99).<sup>206</sup>

Finally, to examine if competing risk of death may have affected the studied associations, we examined the association between midlife cognitive and physical activity and risk of all-cause mortality. In a fully adjusted model, midlife cognitive (HR 0.83; 95% CI 0.69–0.99) and physical (HR 0.70; 95% CI 0.57–0.87) activity were associated with reduced risk of all-cause mortality.

## 5.3 RESULTS OF PAPER III

**Summary results for Paper III, based on results published in Najjar et al. Reproductive period and dementia: a 44-year longitudinal population study of Swedish women. *Alzheimer's Dement.* 2020.**

**Mean (SD) follow-up time:** 27 years (10 years)

**Mean (SD) age at baseline:** 53 years (6 years)

**Person-years of follow-up:** 36,579

**N of all-cause dementia cases:** 291

**N of AD cases:** 146

**N of dementia with CVD cases:** 116

**Mean age at dementia onset:** 80 years (8 years)

**N censored due to death:** 973

**Median age at death (min, max):** 79 years (43, 98 years)

**Mean length of reproductive period (SD) for women with natural menopause:** 35 years (5 years)

**Mean age at menopause (SD) for women with natural menopause:** 49 years (4 years)

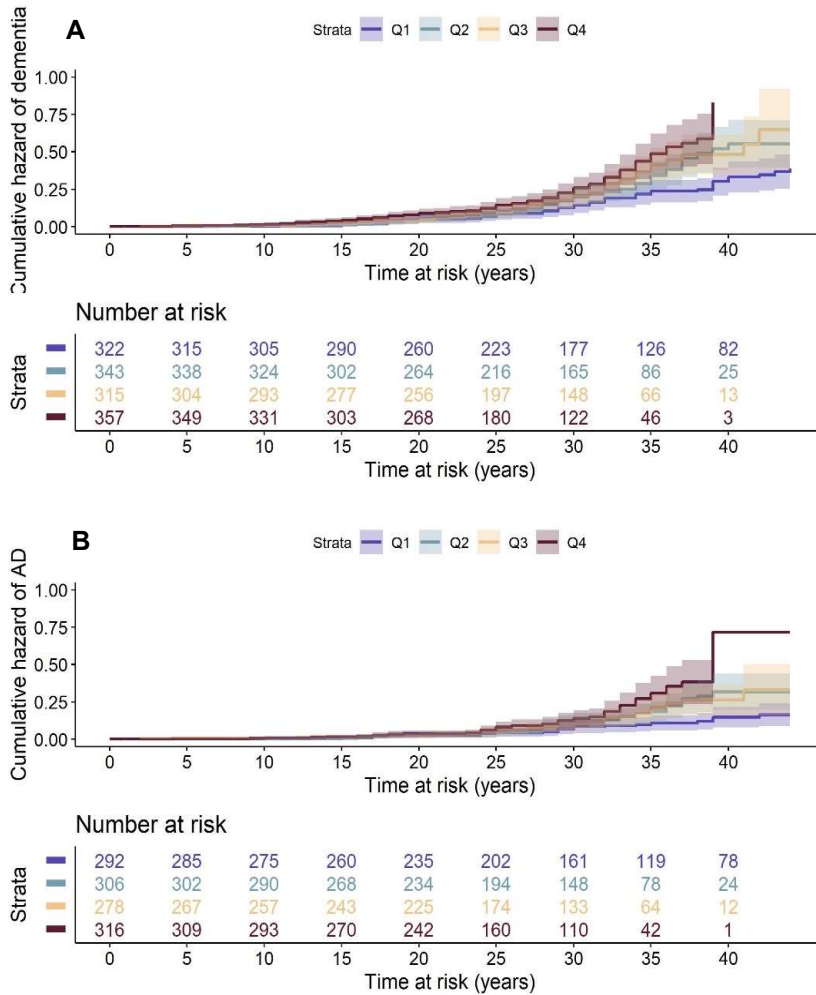
Sociodemographic factors and sample characteristics of *Paper III* are shown in Table 4. Women in higher quartiles of reproductive period had higher age at baseline ( $P<.001$ ), earlier age at menarche ( $P<.001$ ), later age at menopause ( $P<.001$ ), longer duration of breastfeeding ( $P=.02$ ), higher educational attainment ( $P=.01$ ), and were less often smokers ( $P<.001$ ) compared to women in lower quartiles of reproductive period.<sup>207</sup>

Longer reproductive period was associated with increased risk of all-cause dementia (HR per increased year 1.06; 95% CI 1.03–1.20, model 3) and AD (HR per increased year 1.06; 95% CI 1.02–1.11, model 3) in model 1–3.<sup>207</sup> The greatest difference was observed between the 4<sup>th</sup> quartile and the 1<sup>st</sup> quartile of reproductive period (for all-cause dementia, model 3: HR 2.17; 95% CI 1.51–3.11; for AD, model 3: HR 2.78; 95% CI 1.65–4.71; Figure 18).<sup>207</sup> Further, later age at menopause was associated with increased risk of dementia (model 3: HR per increased year 1.07; 95% CI 1.04–1.10) and AD (model 3: HR per increased year 1.07; 95% CI 1.02–1.12) in model 1–3.<sup>207</sup> The results did not change after excluding those who developed dementia before 2000 ( $n=144$ ) and after adjusting for *APOE ε4* allele (subsample of women with genotyping data  $n=603$ ).<sup>207</sup>

**Table 4.** Sociodemographic and health characteristics by quartiles of reproductive period in women with natural menopause (N=1,364).

Characteristics	Quartiles of reproductive period			
	1 <sup>st</sup> Quartile	2 <sup>nd</sup> Quartile	3 <sup>rd</sup> Quartile	4 <sup>th</sup> Quartile
Age (year), Median (min, max)	47 (38, 70)	52 (46, 66)	54 (50, 74)	58 (50, 78)
Reproductive period (year), Mean (SD)	29 (3)	34 (1)	37 (1)	40 (2)
Age at menarche (year), Mean (SD)	14 (2)	14 (1)	14 (1)	13 (1)
Age at menopause (year), Mean (SD)	43 (3)	48 (1)	50 (1)	53 (2)
Pregnancies (number of), Median (min, max)	2 (0, 8)	2 (0, 8)	2 (0, 9)	2 (0, 11)
Breastfeeding (months), Median (min, max)	7 (0, 60)	6 (0, 56)	7 (0, 84)	8 (0, 48)
Oral contraceptives (used), % (cases/total number)	8 (27/333)	11 (39/347)	10 (31/320)	12 (45/364)
HRT (used), % (cases/total number)	5 (17/333)	4 (12/347)	4 (14/320)	7 (24/364)
Exogenous estrogen (used), % (cases/total number)	11 (37/333)	13 (46/347)	13 (43/320)	18 (64/364)
Education* (>compulsory), % (cases/total number)	24 (79/331)	29 (101/346)	32 (102/319)	36 (131/364)
Psychological stress <sup>§</sup> (frequent or constant stress), % (cases/total number)	39 (129/331)	32 (110/346)	35 (110/319)	37 (134/364)
APOE genotype <sup>  </sup> (at least one ε4 allele), % (cases/total number)	28 (33/116)	26 (37/142)	32 (49/154)	31 (60/191)
Physical activity <sup>†</sup> (active), % (cases/total number)	80 (260/326)	80 (274/344)	82 (260/316)	85 (303/357)
Smoking status (former or current smokers), % (cases/total number)	54 (180/333)	53 (184/347)	49 (157/320)	37 (135/364)
Waist-Hip-Ratio <sup>‡</sup> (>0.74), % (cases/total number)	56 (184/329)	56 (192/346)	59 (188/319)	56 (202/364)
Hypertension, % (cases/total number)	21 (69/333)	21 (72/347)	20 (64/320)	22 (80/364)
Diabetes Mellitus, % (cases/total number)	1 (4/333)	1 (2/347)	1 (2/320)	1 (2/364)
Ischemic heart disease, % (cases/total number)	23 (77/333)	19 (67/347)	20 (65/320)	20 (72/364)

Source: Table 1 in Najjar et al. 2020.<sup>207</sup> with permission from Wiley Periodicals LLC, adapted by the author. \*n=1,360. <sup>§</sup>n=1,360. <sup>||</sup>Subsample of 603 women with information on endogenous estrogen exposures and APOE carriership. <sup>†</sup>n=1,343. <sup>‡</sup>n=1,348.



**Figure 18.** Cumulative hazard of (A) all-cause dementia and (B) Alzheimer’s disease (AD), by quartiles of reproductive period (Q1–Q4). The y-axis depicts the cumulative hazard, and the x-axis depicts time at risk shown in years. Analyses adjusted for birth year, number of pregnancies, months of breastfeeding, physical activity, WHR, hypertension, ischemic heart disease, and psychological stress set to sample average. Quartiles of reproductive period are demonstrated in different colors, with shaded areas showing the 95% confidence intervals. Source: Original by the author, based on the results published Najjar et al. 2020.<sup>207</sup>

No association was observed between age at menarche, number of pregnancies, and months of breastfeeding and incident dementia (see Table 2 in *Paper III*). Further, no association was observed between indicators of endogenous estrogen and risk of dementia with CVD (see supplementary table 1 for *Paper III*).<sup>207</sup>

After stratifying by age at dementia onset, we found that longer reproductive period and later menopause were associated with incident all-cause dementia and AD after age 75, with the strongest association in those with all-cause dementia and AD onset after age 85 (Table 5).<sup>206</sup>

**Table 5.** Length of reproductive period and age at menopause in relation to incident dementia and AD in a fully adjusted model (model 3), stratified by different ages of onset.

	<b>Total dementia</b>	<b>Alzheimer's disease</b>
<b>Age at onset &lt;65 years (dementia cases n=13, AD cases n=7)</b>		
Reproductive period	0.94 (0.83–1.07)*	1.17 (0.57–2.42)*
Age at menopause	0.94 (0.83–1.06)†	0.99 (0.80–2.01)†
<b>Age at onset 65–74 years (dementia cases n=54, AD cases n=20)</b>		
Reproductive period	1.03 (0.96–1.09)*	0.94 (0.62–1.42)*
Age at menopause	1.03 (0.96–1.10)†	0.93 (0.61–1.41)†
<b>Age at onset 75–84 years (dementia cases n=134, AD cases n=71)</b>		
Reproductive period	<b>1.05 (1.01–1.10)*</b>	<b>1.25 (1.00–1.56)*</b>
Age at menopause	<b>1.07 (1.02–1.12)†</b>	1.15 (0.93–1.44)†
<b>Age at onset ≥85 years (dementia cases n=90, AD cases n=48)</b>		
Reproductive period	<b>1.10 (1.04–1.17)*</b>	<b>1.15 (1.06–1.26)*</b>
Age at menopause	<b>1.10 (1.03–1.18)†</b>	<b>1.17 (1.07–1.29)†</b>

Source: Table 3 in Najar et al. 2020,<sup>207</sup> with permission from Wiley Periodicals LLC, adapted by the author. Associations are presented as Hazard ratios (95% Confidence intervals). Reproductive period and age at menopause are reported in years. \*Included are: reproductive period, number of pregnancies, months of breastfeeding, birth year, exogenous estrogen, physical activity, WHR, hypertension, ischemic heart disease, and psychological stress. †Included are: age at menarche, age at menopause, number of pregnancies, months of breastfeeding, birth year, psychological stress, and hypertension. Results that surpasses  $P < .05$  are bolded.

To examine if the studied associations were affected by the competing risk of death, we examined the association between reproductive period and age at menopause and risk of all-cause mortality. Longer reproductive period (HR per increased year 1.04; 95% CI 1.03–1.06) and later age at menopause (HR per increased year 1.05; 95% CI 1.03–1.07) were associated with increased risk of all-cause mortality in model 3.<sup>207</sup>

## 5.4 RESULTS OF PAPER IV

### **Summary results for *Paper IV*.**

**Mean (SD) follow-up time:** 20 years (4 years)

**Median (min, max) age at baseline:** 52 years (46, 60 years)

**Median (min, max) age at LP:** 74 years (70, 85 years)

**Mean age (SD) at menarche:** 14 (2 years)

**Mean age (SD) at menopause for women with natural menopause:** 49 years (4 years)

**Mean reproductive period (SD) for women with natural menopause:** 35 years (4 years)

As this paper has not been published, this section will only include a short summary of the results. A more detailed description of the results can be found in *Paper IV*.

In summary, longer reproductive period was associated with lower levels of A $\beta$ 42, lower ratio of A $\beta$ 42/A $\beta$ 40, and higher levels of P-tau, while no association was observed between length of reproductive period and levels of T-tau. In a separate analysis, earlier age at menarche was associated with higher levels of P-tau and lower ratio of A $\beta$ 42/A $\beta$ 40, while no association was observed between age at menopause and levels of CSF biomarkers for AD.



## 5.5 RESULTS OF PAPER V

**Summary results for Paper V, based on results published in Najjar et al. Polygenic risk scores for Alzheimer's disease are related to dementia risk in APOE  $\epsilon 4$  negatives. *Alzheimers Dement (Amst)*. 2021.**

**Mean (SD) follow-up time:** 7 years (5 years)  
**Mean (SD) age at blood sampling:** 80 years (10 years)  
**Person-years of follow-up:** 14,775  
**N of dementia cases:** 605  
**Mean age at dementia onset:** 89 years (8 years)  
**N censored due to death:** 1,243  
**Median age at death (min, max):** 90 years (71, 111 years)

Sample characteristics are shown in Table 1 of *Paper V*. Compared to *APOE*  $\epsilon 4$  non-carriers,  $\epsilon 4$  carriers had a lower age at blood sampling (mean age 79 vs 81 years,  $P < .0001$ ), earlier age at all-cause dementia onset (mean age 87 vs 90 years,  $P < .0001$ ), and earlier age at death (median age 89 vs 91 years,  $P < .0001$ ). Compared to the low-risk tertile of the 39-SNPs AD-PRS, middle- and high-risk tertiles were less likely *APOE*  $\epsilon 4$  carriers (49% vs 19% and 9%,  $P < .0001$ ). Also, middle- and high-risk tertiles of  $1e^{-5}$  AD-PRS had earlier age at all-cause dementia onset than the  $1e^{-5}$  AD-PRS low-risk tertile (mean age 90 vs 89 years,  $P = .04$ ).

In the total sample ( $n = 2,052$ ), *APOE*  $\epsilon 4$  carriership was associated with increased risk and  $\epsilon 2$  carriership with reduced risk of all-cause dementia in fully adjusted models (Table 6).<sup>208</sup>

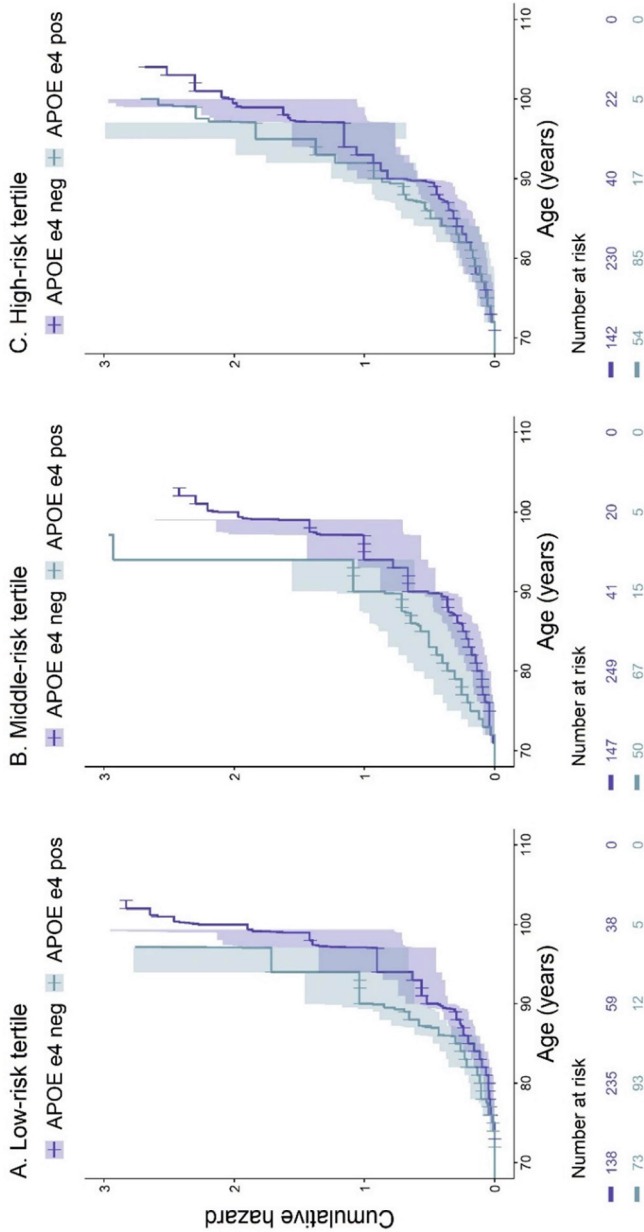
We found an interaction between *APOE*  $\epsilon 4$  carriership and the 39-SNPs AD-PRS ( $P = .02$ ), and between  $\epsilon 4$  carriership and the  $1e^{-5}$  AD-PRS ( $P = .05$ ) in relation to risk of all-cause dementia, while no interaction was seen between  $\epsilon 4$  carriership and the  $1e^{-3}$  AD-PRS or the  $1e^{-1}$  AD-PRS in relation to incident all-cause dementia.<sup>208</sup> Further, no interaction was observed between *APOE*  $\epsilon 2$  carriership and AD-PRSs in relation to incident all-cause dementia.<sup>208</sup>

Based on the interaction analyses, we investigated the effect of *APOE*  $\epsilon 4$  carriership on incident all-cause dementia stratified by the 39-SNPs AD-PRS and the  $1e^{-5}$  AD-PRS.<sup>208</sup> In the total sample, *APOE*  $\epsilon 4$  carriership was associated with increased risk of dementia only in the low- and middle risk tertiles of AD-PRSs (Figure 19, Table 6).<sup>208</sup> The results were similar in those aged 70–94 years, with the exception that  $\epsilon 4$  carriership was associated with increased risk of dementia in all tertiles of the  $1e^{-5}$  AD-PRS (Table 6).<sup>208</sup> In those aged 95 years or older, *APOE*  $\epsilon 4$  carriership was associated with incident all-cause dementia only in the low-risk tertile of AD-PRSs (Table 6).<sup>208</sup>

**Table 6.** Relationship between *APOE*  $\epsilon 4$  carriership and risk of all-cause dementia, stratified by tertiles of AD-PRSs, presented in the total sample, in those aged 70–94 years, and in those aged 95 years or older.

	HR	Confidence interval	P value
<b>Total sample (n=2,052)</b>			
<i>APOE</i> $\epsilon 4$ carriership	1.60	1.35–1.92	<b>1×10<sup>-7</sup></b>
$\epsilon 4$ carriership in low-risk tertile of 39-SNPs AD-PRS	1.98	1.47–2.66	<b>7×10<sup>-6</sup></b>
$\epsilon 4$ carriership in middle-risk tertile of 39-SNPs AD-PRS	2.00	1.43–2.81	<b>6×10<sup>-5</sup></b>
$\epsilon 4$ carriership in high-risk tertile of 39-SNPs AD-PRS	1.18	0.75–1.84	.48
$\epsilon 4$ carriership in low-risk tertile of 1e <sup>-5</sup> AD-PRS	2.20	1.62–3.00	<b>5×10<sup>-7</sup></b>
$\epsilon 4$ carriership in middle-risk tertile of 1e <sup>-5</sup> AD-PRS	1.41	1.01–1.96	<b>.04</b>
$\epsilon 4$ carriership in high-risk tertile of 1e <sup>-5</sup> AD-PRS	1.35	0.99–1.82	.05
<b>70–94 years (n=1,717)</b>			
<i>APOE</i> $\epsilon 4$ carriership	1.75	1.42–2.16	<b>2×10<sup>-7</sup></b>
$\epsilon 4$ carriership in low-risk tertile of 39-SNPs AD-PRS	2.01	1.37–2.95	<b>4×10<sup>-4</sup></b>
$\epsilon 4$ carriership in middle-risk tertile of 39-SNPs AD-PRS	2.39	1.61–3.55	<b>2×10<sup>-5</sup></b>
$\epsilon 4$ carriership in high-risk tertile of 39-SNPs AD-PRS	1.65	0.99–2.77	.06
$\epsilon 4$ carriership in low-risk tertile of 1e <sup>-5</sup> AD-PRS	2.13	1.48–3.08	<b>6×10<sup>-5</sup></b>
$\epsilon 4$ carriership in middle-risk tertile of 1e <sup>-5</sup> AD-PRS	1.87	1.26–2.77	<b>2×10<sup>-3</sup></b>
$\epsilon 4$ carriership in high-risk tertile of 1e <sup>-5</sup> AD-PRS	1.44	1.01–2.06	<b>.04</b>
<b>≥95 years (n=335)</b>			
<i>APOE</i> $\epsilon 4$ carriership	1.33	0.94–1.88	.11
$\epsilon 4$ carriership in low-risk tertile of 39-SNPs AD-PRS	1.72	1.01–2.92	<b>.05</b>
$\epsilon 4$ carriership in middle-risk tertile of 39-SNPs AD-PRS	1.27	0.55–2.95	.58
$\epsilon 4$ carriership in high-risk tertile of 39-SNPs AD-PRS	0.79	0.30–2.09	.63
$\epsilon 4$ carriership in low-risk tertile of 1e <sup>-5</sup> AD-PRS	3.66	1.99–6.73	<b>3×10<sup>-5</sup></b>
$\epsilon 4$ carriership in middle-risk tertile of 1e <sup>-5</sup> AD-PRS	0.71	0.35–1.43	.3
$\epsilon 4$ carriership in high-risk tertile of 1e <sup>-5</sup> AD-PRS	1.06	0.55–2.02	.9

Source: Table 3 in Najar et al. 2021,<sup>208</sup> with permission from Wiley Periodicals LLC, adapted by the author. All analyses are adjusted for age, sex, and 10 principal components to correct for population stratification. Results that surpasses  $P < .05$  are bolded.



**Figure 19.** Cumulative hazard of all-cause dementia by *APOE*  $\epsilon 4$  carriership (*APOE*  $\epsilon 4$  non-carriers are defined as *APOE*  $\epsilon 4$  neg and *APOE*  $\epsilon 4$  carriers are defined as *APOE*  $\epsilon 4$  pos), stratified by tertile of the  $1e^{-5}$  AD-PRS based on the total sample ( $n=2,052$ ). Source: Original by the author, based on the results from Najar et al. 2021.<sup>208</sup> Analyses adjusted for covariates (age at blood sampling, birth year, sex, and 10 principal components to correct for population stratification) set to sample average.

Among the AD-PRSs, only the  $1e^{-5}$  AD-PRS was associated with increased risk of all-cause dementia in the total sample (Table 7).<sup>208</sup> Based on the interaction analyses, we investigated the effect of AD-PRSs stratified by *APOE*  $\epsilon 4$  carriership.<sup>208</sup> Both AD-PRSs (the  $1e^{-5}$  AD-PRS and the 39-SNPs AD-PRS) were associated with increased risk of all-cause dementia in *APOE*  $\epsilon 4$  non-carriers, while no association was observed in  $\epsilon 4$  carriers (Table 7).<sup>208</sup> In those aged 70–94 years, the 39-SNPs AD-PRS was associated with increased risk of all-cause dementia only among *APOE*  $\epsilon 4$  non-carriers, while the  $1e^{-5}$  AD-PRS was not associated with dementia risk in this age group (Table 7).<sup>208</sup> In those aged 95 years or older, the  $1e^{-5}$  AD-PRS was associated with increased risk of all-cause dementia, while the 39-SNPs AD-PRS was associated with increased risk of all-cause dementia in *APOE*  $\epsilon 4$  non-carriers and reduced risk of dementia in *APOE*  $\epsilon 4$  carriers (Table 7).<sup>208</sup>

**Table 7.** Relationship between AD-PRSs (the 39-SNPs-, and the  $1e^{-5}$  AD-PRS) and risk of all-cause dementia, stratified by *APOE*  $\epsilon 4$  carriership, presented in the total sample, in those aged 70–94 years, and in those aged 95 years or older.

	HR	Confidence interval	P value
<b>Total sample (n=2,052)</b>			
39-SNPs AD-PRS	1.03	0.95–1.11	.5
39-SNPs AD-PRS in <i>APOE</i> $\epsilon 4$ non-carriers	1.22	1.10–1.35	<b><math>2 \times 10^{-4}</math></b>
39-SNPs AD-PRS in <i>APOE</i> $\epsilon 4$ carriers	0.94	0.79–1.12	.5
$1e^{-5}$ AD-PRS	1.09	1.01–1.19	<b>.03</b>
$1e^{-5}$ AD-PRS in <i>APOE</i> $\epsilon 4$ non-carriers	1.15	1.05–1.27	<b><math>4 \times 10^{-3}</math></b>
$1e^{-5}$ AD-PRS in <i>APOE</i> $\epsilon 4$ carriers	0.94	0.81–1.09	.4
<b>70–94 years (n=1,717)</b>			
39-SNPs AD-PRS	0.99	0.89–1.09	.8
39-SNPs AD-PRS in <i>APOE</i> $\epsilon 4$ non-carriers	1.16	1.01–1.34	<b>.03</b>
39-SNPs AD-PRS in <i>APOE</i> $\epsilon 4$ carriers	1.08	0.88–1.33	.5
$1e^{-5}$ AD-PRS	1.07	0.96–1.19	.2
$1e^{-5}$ AD-PRS in <i>APOE</i> $\epsilon 4$ non-carriers	1.18	0.98–1.27	.1
$1e^{-5}$ AD-PRS in <i>APOE</i> $\epsilon 4$ carriers	0.99	0.83–1.17	.9
<b>≥95 years (n=335)</b>			
39-SNPs AD-PRS	1.12	0.98–1.29	.1
39-SNPs AD-PRS in <i>APOE</i> $\epsilon 4$ non-carriers	1.28	1.10–1.50	<b><math>2 \times 10^{-3}</math></b>
39-SNPs AD-PRS in <i>APOE</i> $\epsilon 4$ carriers	0.62	0.41–0.95	<b>.03</b>
$1e^{-5}$ AD-PRS	1.15	1.01–1.32	<b>.04</b>
$1e^{-5}$ AD-PRS in <i>APOE</i> $\epsilon 4$ non-carriers	1.12	0.98–1.27	.1
$1e^{-5}$ AD-PRS in <i>APOE</i> $\epsilon 4$ carriers	0.75	0.52–1.08	.1

Source: Table 2 in Najar et al. 2021,<sup>208</sup> with permission from Wiley Periodicals LLC, adapted by the author. All analyses are adjusted for age, sex, and 10 principal components to correct for population stratification. Results that surpasses  $P < .05$  are bolded.

Moreover, the  $1e^{-3}$  and the  $1e^{-1}$  AD-PRSs were not associated with risk of dementia (Supplementary table 2 for Paper V).<sup>208</sup>

Finally, to examine the effect of competing risk of death on the studied associations, we investigated the association between *APOE* genotype, and AD-PRSs (the 39-SNPs AD-PRS and the  $1e^{-5}$  AD-PRS) and risk of all-cause mortality.<sup>208</sup> *APOE*  $\epsilon 4$  carriership was associated with increased risk of all-cause mortality (HR 1.21; 95% CI 1.07–1.39,  $P = 3 \times 10^{-3}$ ), whereas no association was found for  $\epsilon 2$  carriership and the AD-PRSs.<sup>208</sup>

## 5.6 MISSING DATA

In this thesis, we did not perform any type of imputation of missing data. Differences between those included in the analytic sample and those excluded were analyzed using analysis of variance (ANOVA) for differences in means, independent-sample Kruskal-Wallis test and the Mann-Whitney U test for medians, and  $\chi^2$  test for proportions.

*Paper I:* Results regarding differences between those included in the analytic sample of the H70-studies (n=913) and the MCSA 70+ study (n=3,471) and those excluded (the H70-studies: n=24, the MCSA 70+ study: n=56; Figure 8) can be found in *Paper I*.

*Paper II:* Those included in the analytic sample (n=784) had a higher SES compared to those excluded (n=16, Figure 9), while no differences was found for educational attainment, BMI, hypertension, smoking, diabetes mellitus, psychological stress, and major depression. It should be noted, however, that some analyses could be underpowered to find differences between the groups.

*Paper III:* Those included in the analytic sample (n=1,364) had a lower median age at death ( $P=.01$ ), lower mean WHR ( $P<.001$ ), and more psychological stress ( $P=.03$ ) compared to those excluded (n=92, Figure 9).

*Paper IV:* LP participants (n=88) were younger, had higher education, developed dementia less often after follow-up examinations, and had a lower 5-year mortality rate compared to non-participants (n=502), as described previously.<sup>222</sup> Results regarding differences in reproductive history between LP participants and nonparticipants and differences between those included in the analytic sample (n=75) and those excluded (n=13, Figure 9) can be found in *Paper IV*.

*Paper V:* Those included in the analytic sample (n=2,052) had a higher mean age at baseline ( $P<.001$ ), were more likely women ( $P<.001$ ), and had a lower frequency of *APOE*  $\epsilon 4$  carriership ( $P<.001$ ) compared to those excluded (n=1131, Figure 9).<sup>208</sup>

*“Research is formalized curiosity. It is poking and prying with a purpose.”*

Zora Neale Hurston

## 6 DISCUSSION

### 6.1 LIFESTYLE FACTORS AND DEMENTIA

In this thesis, we reported that lifestyle factors, particularly marital status and leisure time cognitive and physical activity were associated with risk of all-cause dementia and dementia subtypes.

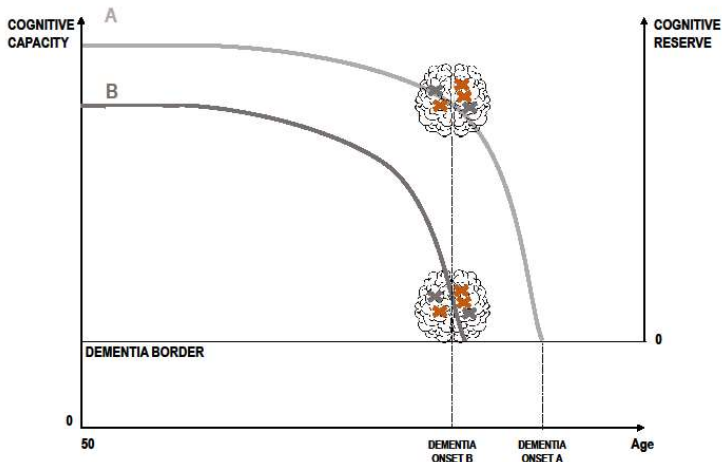
In *Paper I*, in two population-based samples from Rochester (MN), USA, and Gothenburg, Sweden, we found that married men had a reduced risk of dementia compared to unmarried men, while no association was observed between marital status and risk of all-cause dementia among women. However, as can be seen in the result section of *Paper I*, there were some differences in the findings between the MCSA 70+ study and the H70-studies. Reasons for divergent results could be differences in the proportions of sex and marital status between the studies. Compared to the MCSA 70+ study, the H70-studies had a lower proportion of men, married, widowed, as well as a higher proportion of divorced.

In *Paper II*, in a population-based sample of women followed for 44 years, we found that midlife cognitive and physical activity, independently, were associated with reduced risk of different dementia disorders; midlife cognitive activity was associated with reduced risk of all-cause dementia and AD, while midlife physical activity was associated with reduced risk of mixed dementia and dementia with CVD.<sup>206</sup>

Our findings from *Paper I* and *Paper II* are supported by studies reporting an effect of marital status,<sup>84,87-95,97,98</sup> especially among men,<sup>86,91</sup> and leisure time cognitive and physical activity, on cognitive decline and risk of dementia.<sup>82,83,106-119</sup> However, in contrast, two reports from the Whitehall II study and a co-twin study from Sweden reported no relation between midlife leisure cognitive<sup>121</sup> and physical activity<sup>112,120</sup> and risk of dementia. Reasons for discrepant results could be differences in the assessment of dementia diagnosis and study setting. For example, dementia diagnosis in our study and the co-twin study was based on examinations, while the Whitehall II studies used electronic health records. Further, we used a population-based study of women, while the co-twin study used a co-twin sample of men with strict control for genetics and early life exposures.

A possible explanation for our findings that marital status and cognitive activity were associated with risk of dementia might be due to the effect of these lifestyle factors on the cognitive reserve. There are two different forms of reserves that are proposed to compensate for dementia pathology: the brain reserve and the cognitive reserve.<sup>206,257</sup> While the brain reserve refers to quantitative measures, such as the brain size and

neuronal count, the cognitive reserve refers to the ability to make use of the brain reserve.<sup>257</sup> The theory of the cognitive reserve is that people with higher cognitive reserve require more dementia pathology before clinical symptoms of dementia appear (e.g., greater decrease in cortical thickness and regional atrophy, and more amyloid pathology) (Figure 20).<sup>257</sup>



**Figure 20.** Illustration of the cognitive reserve theory. Source: Original by the author, based on Tucker and Stern 2011.<sup>257</sup> The left Y-axis depicts level of cognitive capacity and the right Y-axis depicts level of cognitive reserve. The first X-axis depicts age and the second X-axis depicts dementia border. Person A (light grey line) had higher cognitive capacity and reserve than Person B (dark grey line). Person B developed dementia earlier than Person A, even though both Person A and Person B (hypothetically) had similar amount of dementia pathology at this specific time.

Support for this comes from the Nun Study reporting that low cognitive ability in early life, measured as linguistic ability, was associated with increased risk of AD more than half a century later and AD pathology post mortem.<sup>258</sup> Further support comes from a PET study, reporting that higher cognitive activities in early- and midlife was associated with lower brain amyloid pathology later in life.<sup>259</sup> There is also evidence that marital status could affect the cognitive reserve; the Rush Memory and Aging Project reported that cognitively normal people with larger social networks performed better on cognitive tests despite having similar amount of dementia pathology as individuals with smaller networks.<sup>260</sup> Further, our finding that marital status was associated with incident dementia, particularly in men, could be explained by sex differences in the experience of loneliness. Men who are not in a relationship may experience loneliness to a higher degree than single women may; previous studies report that married men rely more exclusively on their partner for social support whereas married women have larger social networks of friends and relatives to rely



on.<sup>91,261-263</sup> Furthermore, loneliness is suggested to activate stress responses with downstream effects on cognition, mediated by sleep disturbance, dysregulation of the immune system, increased oxidative stress, and decreased levels of brain-derived neurotrophic factors.<sup>264-267</sup> Another explanation for our finding that married men had a reduced risk of dementia compared to unmarried men could be the association between marital status and other illnesses and health measures than dementia. In support of this, studies have reported that married men had a reduced risk of hypertension,<sup>105</sup> depression,<sup>104</sup> all-cause mortality, and mortality due to cancer and cardiovascular disease<sup>268</sup> compared to unmarried men and women. Indeed, in both samples of the present study, we found that not married men had an increased risk of all-cause mortality compared to married men, while no association was observed in women.

Moreover, our finding that midlife physical activity was associated with reduced risk of mixed dementia and dementia with CVD but not with AD,<sup>206</sup> is supported by a recent study reporting a reduced risk of VaD in Vasaloppet skiers compared to non-skiers.<sup>118</sup> In a sample from the Malmo Diet and Cancer Study, the same study reported a reduced risk of VaD in individuals with higher physical activity.<sup>118</sup> Furthermore, the study found no relation between voluntary running and memory improvement, A $\beta$ , or synaptic proteins, in AD mice.<sup>118</sup> Further support comes from a PET study that did not find an association between physical activity and amyloid pathology in the brain.<sup>259</sup> Instead, the effect of midlife physical activity on mixed dementia and dementia with CVD could be attributable to cardiovascular risk factors and stroke. We did not find an association between midlife physical activity and VaD. However, this may be explained by low statistical power since the effect sizes were in the same direction.<sup>206</sup>

Finally, marital status and cognitive and physical activity were associated with risk of all-cause mortality, which most likely attenuated the examined associations. This demonstrate the importance of considering the competing risk of death in studies examining lifestyle factors in relation to risk of dementia.

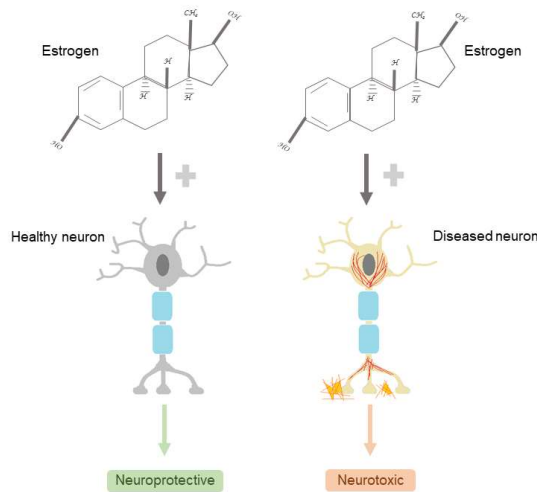
## 6.2 INDICATORS OF ENDOGENOUS ESTROGEN AND DEMENTIA

We also found that longer reproductive period and later age at menopause, as indicators of endogenous estrogen, were associated with increased risk of all-cause dementia and AD, especially after age 85 years.<sup>207</sup> Adding to this, we found that longer reproductive period was associated with CSF biomarkers for AD in the preclinical phase of AD.

Support for our findings comes from the population-based Rotterdam study, reporting that higher levels of estradiol in serum, longer reproductive period, and later age at menopause were associated with increased risk of dementia.<sup>146,152</sup> Further support

comes from neuroimaging studies reporting smaller hippocampal volumes<sup>147</sup> and reduced total brain volumes<sup>165</sup> in women with longer reproductive period compared to those with shorter. However, our findings are not supported by results from the KP study, showing an increased risk of dementia in women with shorter reproductive period, and the NHIS study reporting a reduced risk of dementia in women with longer reproductive period.<sup>141,153</sup> In addition, the 10/66 study found no association between reproductive period and dementia risk.<sup>154</sup> Differences in study settings and designs (e.g., geographical area of study, duration of follow-up, including women with natural menopause or women with all types of menopause, and the assessment of dementia diagnosis) may explain the divergent results (a more detailed discussion is found in the discussion section of *Paper III*). To the best of our knowledge, no previous study has examined the association between reproductive period and levels of CSF biomarkers for AD.

Our results suggest that longer exposure to endogenous estrogen may increase risk of dementia and AD later in life. A possible explanation for our findings could be the theory of the healthy cell, suggesting a protective effect of estrogen on healthy neurons, but a toxic effect if the neurons have been exposed to AD pathology (Figure 21).<sup>269</sup> Women with longer reproductive period have higher ages at menopause and thus greater levels of estrogen at later stages of life when dementia and AD pathologies start to accumulate.<sup>207</sup> Support for this comes from our finding that longer reproductive period was associated with CSF biomarkers for AD in a sample of cognitively normal women. It is also possible that the effect of estrogen changes between different stages of life, which is supported by the timing hypothesis, suggesting a protective effect of HT on dementia if administrated within five years of menopause, while HT later in life increase risk of dementia.<sup>138,139</sup>



**Figure 21.** Simplified illustration of the healthy cell hypothesis. Source: Original by the author, based on Brinton 2005.<sup>269</sup>

In contrast to our findings in *Paper III*, where we found that the association between length of reproductive period and risk of dementia and AD was driven by age at menopause, this was not observed in *Paper IV*. However, as can be seen in the result section of *Paper IV*, the association between age at menopause and levels of CSF biomarkers for AD were similar to that of reproductive period. Thus, absence of a significant relation could be explained by low statistical power. Instead, we found that earlier age at menarche was associated with CSF biomarkers for AD, which is in line with the results of reproductive period.

Furthermore, our finding that length of reproductive period was associated with levels of P-tau and not T-tau could suggest that the effect observed in our study may have occurred in earlier stages of AD before non-AD related neurodegeneration.<sup>47,270</sup> It should also be noted that, given the relatively small sample size, lack of association between length of reproductive period and levels of T-tau could be due to low statistical power.

However, we are still uncertain about the pathophysiological mechanism behind the effect of reproductive period and age at menopause on risk of dementia and AD. Also, as we used indicators for endogenous estrogen and as the reproductive period ranges over a long time span, our findings may be attributable to other factors than estrogen not accounted for in our studies.

### 6.3 GENETIC FACTORS AND DEMENTIA

In this thesis, we investigated the association between AD-PRSs and *APOE* genotype and risk of all-cause dementia in a population-based sample of individuals aged 70–111 years.<sup>208</sup> We found that AD-PRSs (including 39 or 57 SNPs) and *APOE* genotype were associated with risk of all-cause dementia up to very old ages.<sup>208</sup> The association between the AD-PRSs and risk of dementia was particularly strong among *APOE*  $\epsilon 4$  non-carriers.<sup>208</sup> To the best of our knowledge, this is the first study to report an association between genetic variants associated with AD, beyond *APOE* genotype, and risk of dementia in those aged 95 years and older.<sup>208</sup>

Our findings are supported by previous studies in clinical and population-based settings, reporting an association between AD-PRS and risk of dementia.<sup>176,188,190,195,271,272</sup> However, our finding that the association between AD-PRSs and risk of dementia was stronger in *APOE*  $\epsilon 4$  non-carriers are in contrast to the population-based Rotterdam Study reporting a higher risk in  $\epsilon 4$  carriers,<sup>176</sup> and in contrast to clinical studies reporting no modifying effect of *APOE* genotype.<sup>188,190,272</sup> One important reason for our results could be that the participants included in our study had a relatively high mean age at baseline (80 years) compared to previous studies (e.g., mean age at inclusion in the Rotterdam study was 67.5 years). Due to the

increased risk of death and earlier age at dementia onset in *APOE*  $\epsilon 4$  carriers,<sup>273,274</sup> our study could include a selection of healthier  $\epsilon 4$  carriers with genetic and non-genetic characteristics preventing them from developing dementia at the ages studied. Further evidence for this comes from our finding that *APOE*  $\epsilon 4$  carriership was associated with risk of all-cause dementia in the low-risk tertile of AD-PRSs in those aged 95 years and older.<sup>208</sup> Also, in this age group the 39 SNPs AD-PRS was associated with reduced risk of dementia in *APOE*  $\epsilon 4$  carriers, while the AD-PRS was associated with increased risk of dementia in *APOE*  $\epsilon 4$  non-carriers.<sup>208</sup>

Further, we did not find an association between dementia risk and AD-PRSs with more liberal *P* value thresholds (*P* values  $\geq 1e^{-3}$ ). On the contrary, a study using data from the International Genomics of Alzheimer's Project (IGAP) reported that wider AD-PRSs (including genetic variants at *P* value threshold  $\leq 0.5$ ) predicted AD best.<sup>271</sup> However, differences in dementia status (all-cause dementia in our study and AD in the IGAP study) and in the construction of the PRSs ( $R^2$  threshold for LD clumping was stricter in our study [ $R^2=0.001$ ] compared to a more liberal level [ $R^2=0.2$ ] in the IGAP study) could possibly explain the divergent results.

One possible explanation to our finding that genetic variants associated with AD had an effect on incident all-cause dementia could be that the effect might be driven by AD cases, given that approximately two-thirds of those with a dementia diagnosis have AD in Sweden.<sup>10,206</sup> Another explanation could be genetic pleiotropy (i.e., genetic overlap between diseases).<sup>201</sup> This has previously been reported for the *APOE* genotype, which has been associated with several dementia subtypes other than AD (e.g., VaD, DLB, and FTD).<sup>71</sup> In addition, a previous study reported a genetic correlation between AD and DLB that also remained after excluding the *APOE* locus.<sup>275</sup> Another explanation could be that the genetic variants included in the AD-PRSs are involved in other pathophysiological pathways than the amyloidogenic pathway. As seen in Figure 6 the genetic variants associated with AD are involved pathophysiological processes such as the immune response, cholesterol and lipid metabolism, endosomal-vesical recycling, and endocytosis.<sup>57</sup> We also recently reported that the AD-PRS was associated with NfL in CSF in a population-based sample of cognitively normal individuals, especially in individuals who were free from amyloid pathology.<sup>200</sup> In the same study, little or no effect was observed for other AD specific CSF biomarkers (e.g., A $\beta$ 42, T-tau, P-tau, and neurogranin).<sup>200</sup> This could suggest an effect of AD-PRS on aging processes or other neurodegenerative disorders and cerebrovascular disease.<sup>200</sup>

## 6.4 STRENGTHS

This thesis has several methodological strengths. First, all papers were based on prospective population-based studies with long follow-ups, which is an important feature in studies examining risk factors. Second, information on exposures (marital status, cognitive and physical activity, reproductive history, and genetic risk) was obtained through interviews or physical health examinations, performed by experienced and educated research staff, nurses, and medical doctors. Third, nurses, medical doctors, and specialized medical doctors (psychiatrists, neurologists, or geriatrics), performed the neuropsychiatric examinations throughout the observation periods of the studies and several sources of information were used to identify and diagnose dementia based on established diagnostic criteria. Third, we were able to adjust for several potential confounders. Thus, limiting the potential that our results could be explained by unmeasured confounding. Fourth, in *Paper II–IV*, the women were followed from midlife, which made it possible to assess cognitive and physical activity already in midlife, therefore limiting the possibility of preclinical dementia affecting the activity level, and to assess age at menopause close to the actual event, and therefore limiting recall bias. It should also be noted that one purpose of PPSW was to examine women around the age of menopause.<sup>213</sup> Also, in *Paper II* and *III*, the response rates for all the follow-ups were relatively high (>70%). Fifth, a strength of *Paper I* was the use of two population-based samples from different countries, on different continents, increasing the generalizability. Sixth, in *Paper V*, we used a large sample of participants aged 95 years and older, which made it possible to examine the genetic risk of dementia in the oldest old.

## 6.5 LIMITATIONS

There are methodological limitations that also need to be considered. First, cumulative attrition is a problem in longitudinal population-based studies, resulting in a more selected population at end of follow-up. However, this was partly alleviated by the use of medical records and hospital registry to detect and diagnose dementia in those lost to follow-up in PPSW and the H70-studies. Although these sources are less sensitive in detecting dementia,<sup>276</sup> almost all Swedish citizens receive treatment within the public health care system that is covered by the National Patient Register. If anything, underestimation of dementia diagnoses would attenuate the studied associations. Second, there could be unknown dementia cases in the control group. However, our standardized diagnostic procedures, frequent follow-ups, and the use of medical records and registers to detect dementia decreases this risk. Third, although the diagnosis of dementia subtypes in *Paper II* and *III* could be regarded as a strength, it should be emphasized that diagnosis of dementia subtypes based on clinical assessments alone is difficult, especially at older ages when mixed dementia pathologies are more common.<sup>58,59</sup> We therefore defined dementia with mixed

dementia pathology in various ways (mixed dementia and dementia with CVD). Fourth, another limitation in studies with long-term follow-ups is the competing risk of death. Although, the use of Cox regression models partly accounts for this, in *Paper I–III* and *V*, we found that marital status, particularly among men, cognitive and physical activity, reproductive period, age at menopause, and *APOE ε4* carriership were associated with increased risk of all-cause mortality. This most likely weakened the studied associations related to the exposures in all studies included in this thesis. Fifth, not all participants had data on the exposure variables and covariates used in the different papers. In summary, those included in the analytic sample (i.e., with information on exposure variables) had generally better health, higher educational attainment, and less risk factors for dementia (e.g., less likely *APOE ε4* carriers, lower BMI, less often smokers, and less psychological stress) compared to those excluded due to missing information. Thus, it is possible that our samples were healthier than the general population. Sixth, all members of the samples were from Gothenburg, Sweden, or Rochester (MN), USA. Thus, limiting the possibility to generalize our findings to other populations and ethnicities.

### 6.5.1 LIMITATIONS OF THE SPECIFIC PAPERS

In *Paper I*, we collected information on marital status only at baseline, at age of 70+ years. Thus, we did not have information on marital trajectories, which may have affected the results. Further, we lacked information on quality and duration of the marital status and the living situation for those not in a relationship, which could have affected the studied associations. Also, there were some differences in the classification of marital status between the MCSA 70+ study and the H70-study, which could have affected the results; information on those separated was available in the MCSA 70+ study (not available in the H70-study), while information on those live-apart was available in the H70-study (not available in the MCSA 70+ study). Due to the heterogeneity of those live-apart, and as the aim of the study was to examine difference in dementia risk between those who were married/in a marriage-like relationship and those who were not, those live-apart were included in the “not married” group.

In *Paper II*, cognitive and physical activity were only assessed at baseline when the women were in their midlife. Although level of midlife activities tend to continue into old age,<sup>277</sup> evidence show that activities, particularly physical activity, decline in the preclinical phase of dementia.<sup>120,121</sup> However, in a sensitivity analysis excluding those with dementia before 1990, the association between dementia disorders and cognitive and physical activity remained similar, suggesting that our results were not affected by preclinical dementia.<sup>206</sup> In fact, in this analysis, midlife physical activity was also associated with incident all-cause dementia.<sup>206</sup> Further, the assessment of cognitive activity has not been validated. However, the long-term predictive validity of the

instrument could be regarded as acceptable, as our findings are in agreement with most other similar studies, and as cognitive activity was associated with AD but not with VaD and mixed dementia disorders.<sup>206</sup> It should also be emphasized that the co-shared last author of *Paper II*, Tore Hällström, performed all interviews at baseline in 1968–69. Thus, information on activity levels were obtained in a uniform way for all women.

In *Paper III*, we cannot exclude that recall biases could have affected our findings. Although age at menopause was reported close to the actual event, age at menarche, number of pregnancies, and months of breastfeeding were assessed several years after they occurred. This could explain why these variables were not related to incident dementia in our study. However, after excluding those with dementia before 2000, the results did not change, suggesting that our results were, at least, not affected by biased classification due to preclinical dementia.<sup>207</sup> Further, another limitation is that we used proxies for endogenous estrogen exposure and did not measure estradiol in serum. Moreover, as the sex-specific events causing fluctuations in levels of endogenous estrogens also cause variation in other hormones, the results reported could be explained by other hormones than estrogen. Also, the observed association between indicators of endogenous estrogen and dementia risk may be mediated by aging processes in the hypothalamic-pituitary unit.<sup>207,278</sup> Furthermore, we had a crude assessment of exogenous estrogen. We did not have information on type or levels of estrogens in the medications, if the medication included progesterone, and age when these drugs were taken. Considering the timing hypothesis, lack of information when the HT was taken may explain that we did not find an association with risk of dementia in our study.

In *Paper IV*, in addition to the limitations of the exposure variables described in the section above, a limitation of this study is the rather small sample size. Therefore, some analyses could be underpowered to find small differences between groups. In addition, as only 10% of the eligible sample in 1992–93 performed LP, our sample could include a selection of healthier individuals.<sup>222</sup> Another limitation is that we only had information on CSF biomarkers for AD from the examination in 1992–93. Thus, we could not examine the effect of reproductive period on the change of biomarkers for AD in CSF during follow-up. Further, although we adjusted for number of pregnancies, months of breastfeeding, and number of miscarriages, which contributes to variation in levels of endogenous estrogen during the reproductive span, we did not have information on other measures that could affect levels of estrogen, such as length and regularity of the menstrual cycle. This may have affected the validity of reproductive period as a measure of endogenous estrogen.

In *Paper V*, the inclusion of individuals with genetic data, and thus inclusion of individuals who have survived until at least the year of 2000 when the first blood sampling was performed, and the exclusion of participants with no follow-up data and

dementia at baseline, could render a selection of healthier individuals at the ages observed. Further, even though the large amount of people aged 95 years or older in our study could be regarded as a strength, our findings may have been affected by selection bias and should be interpreted as such. Also, the AD-PRSs included SNPs related to AD. We examined the relation between AD-PRSs on incident all-cause dementia, including other dementia subtypes than AD most likely not as strongly associated with the genetic variants included in the AD-PRSs, which could have attenuated the studied associations.



## 6.6 GENERAL DISCUSSION

Considering that the overarching aim of this thesis was to examine risk factors for dementia, the concept of causality also needs to be addressed.

There are several types of causes: necessary and sufficient, necessary but not sufficient, sufficient but not necessary, and neither sufficient nor necessary.<sup>279</sup> The definition of a necessary cause is that the disease will never occur without the cause (e.g., you will not get appendicitis without a vermiform appendix), while the definition of a sufficient cause is that it will inevitably cause the disease (i.e., if you have the sufficient cause the outcome will follow).<sup>279</sup> However, in multifactorial diseases, such as dementia, there are a myriad of factors that contribute to the occurrence of the disease (i.e., risk factors). These factors are neither sufficient nor necessary to cause the disease, which means that sometimes when the factor happens the disease occur, but the disease can also occur without the factor.<sup>279</sup> Thus, preventative strategies that include blocking causes that are neither necessary nor sufficient will not prevent the disease, but prevent some cases of the disease.<sup>280</sup>

However, it is suggested that causal effects cannot be established in epidemiological studies due to the risk of errors related to study design, sample selection and retention, information acquisition, and lack of controlling for confounding and other biases.<sup>280</sup> Instead, experimental studies and RCTs are regarded as the golden standard of study settings examining causal-effects due to their rigorous control of the environment.<sup>280</sup> Still, it needs to be emphasized that such control is no guarantee against errors.<sup>280</sup>

Furthermore, there are certain features of dementia that are hard to account for in experimental and RCT settings. For example, as aforementioned, dementia is a complex disease, probably caused by the interplay between both genetic and non-genetic factors. For obvious reasons, the effects of biological factors (e.g., sex and genetics), environmental factors (e.g., education and pollution), and social factors (e.g., marital status and social networks) are impossible to study in an RCT setting. Although certain aspects of biological, environmental, and social factors could be examined in experimental studies, epidemiological studies are needed to further disentangle their complex interplay.

Another important feature of dementia (particularly AD) that needs to be considered is the relatively long preclinical phase of the disease (20–30 years).<sup>50</sup> This could, for example, make it difficult to find a true association between leisure time activities and dementia risk in RCTs, since it is rather challenging to conduct RCTs with the observation period required to reduce the risk of preclinical dementia affecting the results. Even though observation periods that span over several decades are difficult to achieve in epidemiological studies (the obstacle being economical rather than ethical),

it is not impossible. However, as mentioned in the limitation section, although studies with long observation periods could be regarded as a strength, especially in studies examining risk factors for dementia, it also has its limitations.

In summary, no study is without errors.<sup>280</sup> Rothman and Greenland suggested that in order to understand the validity of scientific evidence (i.e., the causal inference), the study needs to be evaluated and all errors afflicting the study needs to be quantified.<sup>280</sup> Thus, the validity of the study, taking all measurement errors into account, will help distinguish a causal association from a non-causal association.<sup>280</sup>

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## 7 CONCLUDING REMARKS

This thesis challenges the current knowledge of risk factors for dementia and dementia subtypes.

Lifestyle factors, such as marital status and leisure time cognitive and physical activity may affect the risk of dementia and dementia subtypes. Although previous studies have demonstrated that activity levels could decrease as a result of preclinical dementia, and thus increase the risk of reverse causation,<sup>120,121</sup> the use of a population-based sample of women followed over four decades in this thesis reduced that risk. In fact, in sensitivity analyses excluding those with dementia before 1990 (i.e., 22 years after baseline), the results remained similar, if not stronger for at least physical activity that in these analyses also was associated with incident all-cause dementia.<sup>206</sup> Nevertheless, it needs to be emphasized that our findings do not exclude the possibility that low leisure time activity levels could be a sign of preclinical dementia. However, one does not exclude the other; low leisure time cognitive and physical activity in midlife could have a causal relationship with increased risk of dementia and still be a sign of preclinical dementia. Moreover, our finding of a modifying effect of sex on the relation between marital status and dementia, i.e., that married men had a reduced risk of dementia compared to unmarried men, while no association was found in women, add more knowledge to sex differences in risk factors for dementia.

The findings from this thesis also suggest an association between indicators of endogenous estrogens and risk of dementia and AD, which may be evident already at the preclinical stages of the disease. These results increase the current knowledge of the association between estrogens and risk of dementia, and may contribute with further understanding on the increased risk of dementia and AD in women compared to men.

Furthermore, we found an effect of genetic variants associated with AD on risk of dementia beyond the *APOE* genotype.<sup>208</sup> This association persisted in those aged 95 years or older, particularly in *APOE*  $\epsilon 4$  non-carriers.<sup>208</sup> The results indicate that the genetic risk of dementia continues into the oldest old, and increases our knowledge of identifying individuals at increased risk of dementia in the general population.

Finally, we also examined the effect of these factors on risk of all-cause mortality. We found that marital status, cognitive and physical activity, reproductive period and age at menopause,<sup>207</sup> and *APOE*  $\epsilon 4$  carriership<sup>208</sup> were associated with increased risk of all-cause mortality, demonstrating the importance of considering the competing risk of death in studies examining risk factors for dementia.

## 7.1 FUTURE PERSPECTIVES

Future studies need to be performed in population-based samples from different ethnical groups and geographical areas, in order to evaluate the generalizability of our findings.

Further, it is still not clear whether estrogen acts neuroprotective, neurotoxic, or both in relation to dementia risk. Could differences between measures of estrogens (exogenous, serum levels of endogenous estrogen, or indicators of endogenous estrogens) explain the divergent results in studies conducted to this point? Future studies should include well-defined measures of both exogenous and endogenous estrogens to further disentangle the effect of estrogen on dementia risk. Also, in order to properly examine the potential role of estrogen as an explanation to the differences in risk of dementia and AD between men and women, more studies examining the effect of estrogen on risk of dementia in samples including both men and women are needed. Indeed, the Rotterdam Study reported an increased risk of dementia and smaller hippocampal volumes in women with higher levels of total and bioavailable estradiol, while no association was observed between estradiol levels in serum and risk of dementia among men.<sup>146,147</sup> On the contrary, the Honolulu-Asia Aging Study reported an increased risk of cognitive decline and AD in men with higher estrogen levels compared to men with lower levels, while levels of testosterone was not associated with cognition and AD risk.<sup>281</sup>

Furthermore, we found that midlife cognitive and physical activity was associated with reduced risk of dementia and dementia subtypes in women.<sup>206</sup> To generalize our findings to men, studies including both men and women are needed. Also, to minimize the risk of preclinical dementia affecting the results, studies with observation-periods that last longer than the proposed preclinical phase of dementia (20–30 years) are needed.

Finally, the complex interaction between genetic and non-genetic risk factors for dementia should be further investigated. Although we did not find a modifying effect of *APOE*  $\epsilon 4$  allele on the relationship between length of reproductive period and risk of dementia and AD,<sup>207</sup> we have not examined the modifying effect of other genetic variants associated with AD than *APOE* genotype on this association. Also, we did not examine the modifying effect of genetic variants associated with AD on the relationship between leisure time cognitive and physical activity and dementia risk.

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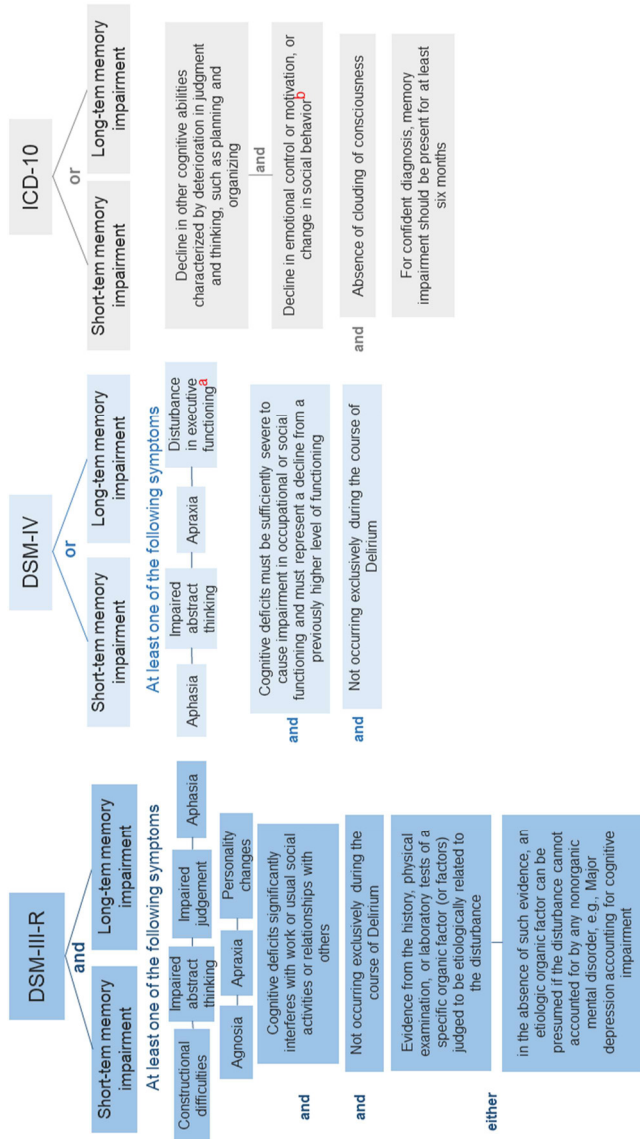


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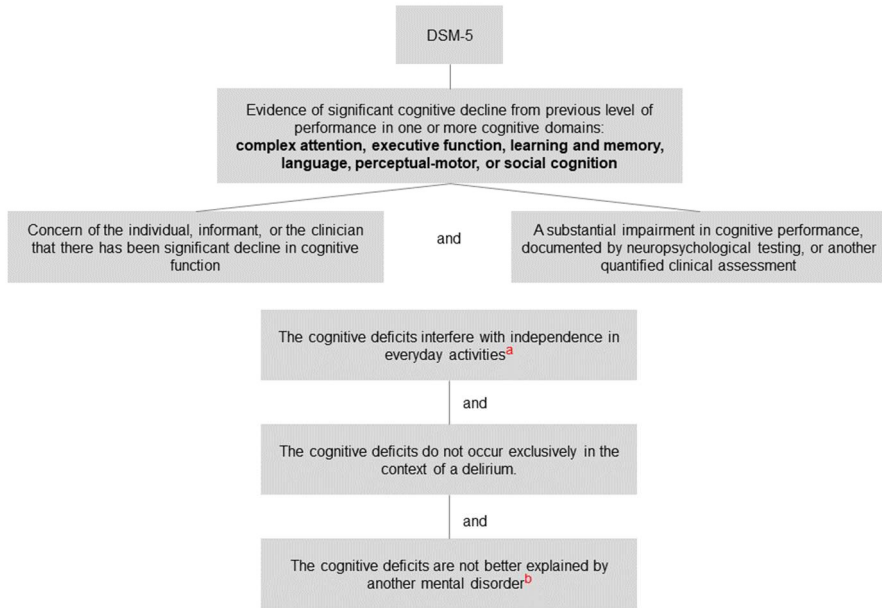
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# APPENDIX 1



Diagnostic criteria of dementia according to DSM-III-R, DSM-IV, and ICD-10. Source: Original by the author, based on information obtained from the Diagnostic and Statistical Manual of Mental Disorders (DSM), Third Edition – Revised, Fourth Edition, American Psychiatric Association, the International Classification of Diseases (ICD), the World Health Organization, and Wancata et al. 2007. <sup>a</sup>Executive functioning such as planning, organizing, sequencing, abstracting. <sup>b</sup>A decline in emotional control or motivation, or a change in social behavior, manifested by at least one of the following: emotional lability, irritability, apathy, and coarsening of social behaviour.

# APPENDIX 2



Diagnostic criteria of Neurocognitive disorder (dementia) according to DSM-5. Source: Original by the author, based on information obtained from the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, American Psychiatric Association, and Sachdev et al. 2014. <sup>a</sup>The cognitive deficits interfere with independence in everyday activities that is, at a minimum, requiring assistance with complex instrumental activities of daily living such as paying bills or managing medications. <sup>b</sup>For example, major depressive disorder or schizophrenia.

## APPENDIX 3

*Diagnostic criteria of Alzheimer's disease (AD) according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association (NINCDS-ADRDA) published in 1984.*

**Diagnosis of probable AD include:**

- dementia established by clinical examination and documented by cognitive examination and neuropsychological test
- deficits in two or more areas of cognition
- progressive worsening of memory and other cognitive functions
- no disturbance in consciousness
- onset between age 40 and 90
- absence of systemic disorder or other brain disease that could explain the progressive decline in memory and cognition

**And is supported by:**

- progressive decline of specific cognitive functions such as aphasia, apraxia, and agnosia
- impaired activities of daily living and altered pattern of behavior
- family history of similar disorders, particularly confirmed neuropathologically
- laboratory results of:
  - o normal lumbar puncture as evaluated by standard techniques
  - o normal pattern or nonspecific changes in EEG (e.g., increased slow-wave activity)
  - o evidence of cerebral atrophy on CT with progression documented by serial observations

**Other clinical features consistent with diagnosis of probable AD, after exclusion of causes of dementia other than AD:**

- plateaus in the course of progression of the disease
- associated symptoms of depression, insomnia, incontinence, delusions, illusions, hallucinations, catastrophic verbal, emotional, or physical outbursts, sexual disorders, and weight loss
- other neurologic abnormalities in some patients, especially with more advanced disease and including motor signs such as increased muscle tone, myoclonus, or gait disorder
- seizures in advanced disease
- CT normal for age

**Features for probable AD is unlikely when:**

- sudden, apoplectic onset
- focal neurological findings such as hemiparesis, sensory loss, visual field deficits, and incoordination early in the course of the illness
- seizures of gait disturbance at the onset or very early in the course of the illness

**Diagnosis of possible AD include:**

- on the basis of dementia syndrome, in the absence of other neurologic, psychiatric, or systemic disorders sufficient to cause dementia, and in the presence of variations in onset, in the presentation, or in the clinical course
- the presence of a second systemic or brain disorder sufficient to produce dementia, which is not considered to be **the** cause of the dementia

# APPENDIX 4

*Diagnostic criteria of vascular dementia (VaD) according to the National Institute of Neurological Disorders and Stroke–Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) published in 1993.*

VaD is characterized by cognitive impairment resulting from ischemic or hemorrhagic stroke or from ischemic-hypoxic brain lesions.

**Diagnosis of probable VaD include:**

1. *Dementia diagnosis* established by clinical examination and neuropsychological testing; deficits should be severe enough to interfere with activities of daily living, not due to physical effects of stroke alone.
2. *Cerebrovascular disease*, defined by the presence of focal signs and neurological examinations (e.g., hemiparesis, lower facial weakness, Babinski sign, sensory deficit, hemianopia, and dysarthria consistent with stroke (with or without history of stroke), and evidence of relevant CVD by brain imaging including multiple large-vessel infarcts or a single strategically placed infarct (angular gyrus, thalamus, basal forebrain, or PCA or ACA territories), as well as multiple basal ganglia and white lacunes or extensive periventricular white matter lesions, or combinations thereof.
3. *A relationship between* 1) and 2), manifested or inferred by the presence of one or more of the following:
  - o onset of dementia within 3 months following a recognized stroke;
  - o abrupt deterioration in cognitive functions;
  - o or fluctuating, stepwise progression of cognitive deficits.

**Clinical features consistent with the diagnosis of probable VaD:**

- early presence of gait disturbance;
- history of unsteadiness and frequent, unprovoked falls;
- early urinary frequent, urgency, and other urinary symptoms not explained by urologic disease;
- pseudobulbar palsy;
- and personality and mood changes, abulia, depression, emotional incontinence, or other subcortical deficits including psychomotor retardation and abnormal executive function

**Features that make the diagnosis of VaD uncertain or unlikely include:**

- early onset memory deficit and progressive worsening of memory and other cognitive functions such as aphasia, apraxia, agnosia, in the absence of corresponding focal lesions on brain imaging;
- absence of focal neurological signs;
- absence of cerebrovascular lesions on brain CT or MRI

**Diagnosis of possible VaD include:**

- dementia with focal neurological signs without information from neuroimaging to confirm CVD;
- or in the absence of clear temporal relationship between dementia and stroke;
- or in patients with subtle onset and variable course (plateau or improvement) of cognitive deficits and evidence of relevant CVD

## APPENDIX 5

Study	Date of ethical approval	DNR
PPSW	1980-03-05	65-80
PPSW	1991-10-09	Ad 65-80
PPSW/H70	1992-05-19	179-92
PPSW/H70	1992-09-21	314-89
PPSW/H70	1999-09-21	S 377-99
PPSW	1999-10-18	Ö 402-99
PPSW/H70	2000-05-23	S 227-00
PPSW	2000-06-16	Ad Ö 402-99 T 199-00
PPSW/H70	2001-03-20	S 069-01
95+ study	2001-10-31	Ad S 328-00 Ö 424-00 T 417-01
95+ study	2002-01-07	Ad 349-96 R 175-98 S 328-00 T 005-02
95+ study	2002-10-14	Ad 349-96 R 175-98 S 328-00 T 378-02
95+ study	2003-10-14	Ad 349-96 R 175-98 S 328-00 T 415-03
PPSW	2004-03-30	T 123-04
PPSW/H70	2004-11-02	T 453-04
H70 (H85)	2008-11-06	607-08
H70 (H85)	2009-01-15	731-08
PPSW/H70	2009-02-23	075-09
95+ study	2009-03-20	T 170-09 Ad 607-08
PPSW/H70	2009-08-05	328-09
PPSW/H70	2011-11-18	T809-11 Ad 069-01
H70 (H88)	2011-11-21	783-11
H70(H88)	2011-12-22	1021-11
H70 (H90)	2013-06-17	388-13
H70	2013-11-21	869-13
PPSW/H70	2015-04-29	131-15
MCSA 70+ study	2020-10-16	14-004401

Registration (DNR) and reference numbers for all examinations included in this thesis.