Ulrika K Eriksson has a Master in Medical Science with a Major in Biomedicine from Karolinska Institutet, Sweden. Her doctoral thesis consists of epidemiological studies of inflammation-associated risk factors for Alzheimer's disease and dementia using data from the Swedish Twin Registry.

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“If you don’t like the road you’re walking,
start paving another one.”

Dolly Parton

ABSTRACT

Alzheimer's disease (AD) is the leading cause of dementia worldwide and a highly debilitating, and deadly, disease. For the majority of AD cases, the cause of the disease is not known. Chronic inflammation has been implicated in AD pathology. The overall objective of this thesis was to study indices of altered peripheral inflammation as risk factors for dementia in general and Alzheimer's disease in particular. The four studies included in this thesis are observational epidemiological studies based on data from the Swedish Twin Registry. Identifying inflammation-associated risk factors for AD could not only provide clues to the etiology of AD but also lead to novel strategies for combating the disease.

In Study I, the atopic inflammatory disorders asthma, eczema and rhinitis were assessed (prior to dementia follow-up) through questionnaires in the 1960s or 1970s. Dementia was ascertained in two different study designs, longitudinally (n = 22,188) and cross-sectionally (n = 7,800). A history of atopy conferred a 16% increased risk of dementia (Hazard Ratio [HR] 1.16, 95% confidence interval 1.01–1.33) in the longitudinal study but could not be replicated in the cross-sectional study, perhaps due to poorer survival in atopic individuals.

In Study II, manifest cardiovascular diseases other than stroke (CVD) were investigated as proxies for a burden of atherosclerosis (i.e. vascular inflammation). CVD information was collected from national registries and dementia was ascertained by clinical evaluations or register-linkage. Results showed that CVD increases the risk of dementia in general, but also of AD specifically, in carriers (but not non-carriers) of the APOE4 allele (HR 2.39, 1.15-4.96). By analyzing twin pairs, we could also show that the association between CVD and dementia is not explained by genetic or early life environmental factors in common to both disorders.

In Study III, we investigated serum levels of antibodies against phosphorylcholine (anti-PC), a novel marker with anti-atherogenic and anti-inflammatory effects. A nested case-control study of incident dementia (serum collected before onset of dementia) was performed to estimate the relative risk of dementia and AD. In addition, a case-control study of prevalent dementia cases (serum collected after dementia onset) was conducted to investigate differences in anti-PC between dementia cases and controls. We found no increased risk of developing AD or dementia due to lower anti-PC levels whereas patients with AD were more likely to belong to the lowest quartile of anti-PC than age- and sex-matched controls, OR 2.70, 1.45-4.99.

In Study IV, we sought to perform a comprehensive study of the inflammatory markers C-reactive protein (CRP) and interleukin-6 (IL6). Almost 4,000 elderly Swedes (1,265 AD cases) were genotyped for a total of 22 tagSNPs. A sub-set of the population had serum measurements of CRP and IL6 and was included in A) a nested case-control study of incident dementia cases, and B) a case-control study of prevalent dementia cases. None of the SNPs or haplotypes were associated with AD or dementia, nor were there associations between CRP or IL6 levels and the risk of future AD or dementia. However, AD cases were more likely to belong to the highest quartile of IL6 (measured on average 5.5 years after dementia onset) than age- and sex-matched controls, OR 2.24 (1.27-3.95).

In conclusion, this thesis shows that there are significant immune alterations in AD and dementia patients compared to non-demented controls. However, indicators of inflammatory burden, other than CVD, appear to have a limited association with the risk of developing dementia and AD late in life.

LIST OF PUBLICATIONS

This thesis is based on the following original articles, which will be referred to in the text by their Roman numerals.

I. **Eriksson UK**, Gatz M, Dickman PW, Fratiglioni L, Pedersen NL.
Asthma, eczema, rhinitis and the risk for dementia.
Dement Geriatr Cogn Disord 2008;25(2):148-156.

II. **Eriksson UK**, Bennet AM, Gatz M, Dickman PW, Pedersen NL.
Non-Stroke Cardiovascular Disease and Risk of Alzheimer's Disease and Dementia
Alzheimer Dis and Assoc Disord (Accepted)

III. **Eriksson UK**, Sjöberg BG, Bennet AM, de Faire U, Pedersen NL, Frostegård J
Low Levels of Antibodies against Phosphorylcholine in Alzheimer's Disease
J Alzheimers Dis (Accepted)

IV. **Eriksson UK**, Bennet AM, Reynolds CA, Hong M-G, Prince JA, Gatz M, Dickman PW, Pedersen NL
Associations of Gene Sequence Variation and Serum Levels of C-reactive protein and Interleukin-6 with Alzheimer's Disease and Dementia
Manuscript

CONTENTS

1 INTRODUCTION 1
2 BACKGROUND 2

A SHORT INTRODUCTION TO EPIDEMIOLOGY 2
ALZHEIMER'S DISEASE AND DEMENTIA 3
INFLAMMATION AND AD 5
INFLAMMATION-ASSOCIATED RISK FACTORS 7
THE TWIN METHOD 10

3 AIMS 12
4 MATERIALS AND METHODS 13

STUDY SETTING 13
DATA SOURCES 13
DEMENTIA AND AD ASSESSMENT 23
BIOLOGICAL MATERIALS 27
STATISTICAL ANALYSES 28
STUDY DESIGN AND EXPOSURE ASSESSMENT 32
Study I: Atopic disorders and Risk for Dementia 32
Study II: CVD and Alzheimer's disease 33
Study III: Antibodies against Phosphorylcholine and dementia 36
Study IV: CRP, IL6 and Alzheimer's disease 38

5 RESULTS AND DISCUSSION 40

Study I: Atopic disorders and Risk for Dementia 40
Study II: CVD and Alzheimer's disease 42
Study III: Antibodies against Phosphorylcholine and dementia 45
Study IV: CRP, IL6 and Alzheimer's disease 48

6 GENERAL DISCUSSION 52
7 CONCLUSIONS 55
8 ACKNOWLEDGEMENTS 56
9 REFERENCES 58

LIST OF ABBREVIATIONS

AD	Alzheimer's disease
ALR	alternating logistic regression
Anti-PC	IgM antibodies to phosphorylcholine
AP	angina pectoris
APOE4	apolipoprotein E ε4 allele
CABG	coronary artery bypass graft
CDR	Causes of Death Register
CLR	conditional logistic regression
CRP	C-reactive protein
CVD	(non-stroke) cardiovascular disease
DNA	deoxyribonucleic acid
DZ	dizygous (twins)
GEE	generalized estimating equations
HR	hazard ratio
ICD	International Classification of Diseases
IL6	interleukin-6
IPT	in-person testing
MI	myocardial infarction
MZ	monozygous (twins
NPR	National Patient Register
NUD	dementia not otherwise specified (demens utan närmare specification)
OR	odds ratio
PTCA	percutaneous transluminal coronary angioplasty
STR	Swedish Twin Registry
sv.	svenska (Swedish)
TRA	twins reared apart
TRT	twins reared together
SNBHW	Swedish National Board of Health and Welfare
VaD	Vascular dementia
vs.	versus (Latin *against*)

1 INTRODUCTION

Dementia is a syndrome that affects memory and other cognitive functions to the extent that it interferes with daily function. There are many conditions that can cause dementia, including neurodegenerative disorders (e.g. Alzheimer's disease and Parkinson's disease), cerebrovascular disease, brain injury, alcohol abuse, metabolic disorders (e.g. B12 deficiency), and certain infections (e.g. HIV).

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder and the leading cause of dementia worldwide, accounting for approximately 60-70 % of all cases.[1-3] AD is a highly debilitating disorder, progressing from minor memory problems to a complete loss of cognitive functions and eventually death. Prevalence increases exponentially with age, affecting a little more than 1 % in the population aged 65-69 years up to as much as 30-40% in the oldest old.[4] Overall prevalence in the population above 65 years of age is estimated to 6-8% and is expected to increase significantly worldwide due to changing demographic profiles with an ever-increasing proportion of elderly.[5]

This thesis deals with dementia in general and AD in particular. There is a great deal of impetus for understanding the mechanisms that lead to clinical AD and discovering modifiable risk factors. Although the neuropathological hallmarks of AD (amyloid plaques and neurofibrillary tangles) are well known, the etiology of AD remains elusive. Only a minute proportion of AD cases are caused by dominant inherited mutations in genes involved in amyloid processing,[6] the vast majority of AD cases are "sporadic", i.e. there are no known factors that can fully explain disease onset. The most well-established factors found to negatively influence the risk of AD in sporadic cases include a close relative with AD,[7] the $\varepsilon 4$ allele of the APOE gene, and a low level of educational attainment.[8-10]

Inflammation was first implicated in AD pathology in the 1990's with the neuropathological finding of activated inflammatory cells surrounding the amyloid plaques and the epidemiological finding that individuals with a high intake of anti-inflammatory drugs had less AD. Identifying inflammation-associated risk factors for AD could provide clues to the etiology of AD and lead to novel strategies for combating the disease.

2 BACKGROUND

A SHORT INTRODUCTION TO EPIDEMIOLOGY

The four studies included in this thesis are observational epidemiological studies. Epidemiology is "the study of the distribution and determinants of disease frequency" or simplified, "the study of the occurrence of disease".[11] Epidemiology is thus not restricted to the study of infectious diseases as sometimes misconceived by the term "epidemic" when referring to infectious outbreaks. Epidemiological studies can be used both for descriptive purposes ("the study of the distribution of disease"), e.g. to quantify the number of individuals affected with dementia in a population, or for etiological purposes ("the study of determinants of disease"), e.g. to investigate if high levels of interleukin-6 increase the risk of developing dementia. For etiological studies the optimal scientific approach would be to randomize study participants to different exposure groups (as is the case in clinical trials). However, in many instances this is not possible due to ethical reasons or practical feasibility and inference about the effect of an exposure on the risk of developing the disease has to be made by observing the data at hand in the population. The studies included in this thesis all had etiological aims, i.e. to quantify the effect of selected risk factors on the relative risk of developing dementia or AD. Here follows a short description of some key concepts in epidemiology that are frequently used in this thesis.

Prevalence is the proportion of individuals in the population that have the disease at a given time point. It is thus a measure of how many are already affected by the disease.

Incidence measures the passage from disease-free to diseased. Incidence thus refers to the number of new cases that occur in a population during a given period of time.

An exposure is a factor that one believes will either increase the risk of disease (a risk factor) or lower the risk of disease (a protective factor). The primary exposures investigated in this thesis are related to inflammation, i.e. atopic disorders, cardiovascular disease, anti-PC, C-reactive protein and interleukin-6.

The outcome is the "end-point" or disease of interest, the "cases". In this thesis the major outcomes of interest are AD and dementia.

The relative risk (RR) of a disease is calculated as the risk of developing the disease in the exposed group divided by the risk of developing the disease in the unexposed group. It is thus a measure of

how much the specific exposure or risk factor in question affects the risk of developing the disease. For example, a RR of 2.0 indicates that the risk factor in question doubles the risk of developing the disease. Hazard ratios (HR) and odds ratios (OR) are often presented as measures of relative risk.

Cohort studies are often referred to as the "gold standard" epidemiological study. A cohort is a group of people followed longitudinally over time. The purpose of a cohort study is to measure the occurrence (the incidence) of the outcome in the exposed and un-exposed groups, respectively, for the calculation of a relative risk. Cohort studies usually give valid results but can be expensive and time consuming and inadequate when studying rare outcomes.

Case-control studies have the same goals as cohort studies but are conducted "in reverse". Instead of defining individuals according to their *exposure status* and then following them for occurrence of disease, individuals are selected based on their *disease status*, i.e. diseased (cases) and non-diseased (controls), and the exposure status is then (usually) assessed retrospectively. Case-control studies are more prone to bias but can, properly conducted, give valid results and offer a less costly and time-consuming way to study risk factors for disease.

Bias is the equivalent of a systematic error. Results from a biased study will not be valid.

Confounding is a central issue for epidemiological studies and relates back to the non-experimental design of observational studies. Confounding can be defined as the systematic confusion, or mixing, of effects between the exposure, the outcome and *another variable*. An example would be a study of the association between grey hair (exposure) and mortality (outcome). Grey hair would appear to be a risk factor for death when in fact the association would be confounded by age ("another variable"), which is associated with both grey hair and mortality. If age is not adjusted for in the analysis, the study would give the biased result that grey hair "kills".

ALZHEIMER'S DISEASE AND DEMENTIA

Clinical symptoms of dementia relate to the affected areas of the brain. In AD the symptoms are caused by a progressive loss of cholinergic function due to neuronal cell death in the hippocampus and cerebral cortex, brain regions involved in thought processing and memory.[12-16] At the microscopic level, the core hallmarks of AD consists of two kinds of protein aggregates, amyloid plaques and hyper-phosphorylated tangles of tau-protein (Figure 1). Amyloid precursor protein (APP) is a transmembrane protein without known function that is constitutively cleaved into peptides during cell metabolism.[17] The amyloidogenic 40 or 42 amino acid (Aβ) peptide is released

after cleavage by β-secretase and γ-secretase enzymes and is usually quickly removed from the brain. However, in the case of overproduction or impaired clearance, Aβ aggregates into extracellular oligomers, fibrils and eventually, plaques.[18] Tau is an intracellular microtubule binding protein that, when hyper-phosphorylated, will cause disassembly of microtubules and thus will impair axonal transport and compromise neuronal and synaptic function.[19-22] Whether tangle formation is a cause or a consequence of the disease is still under debate.

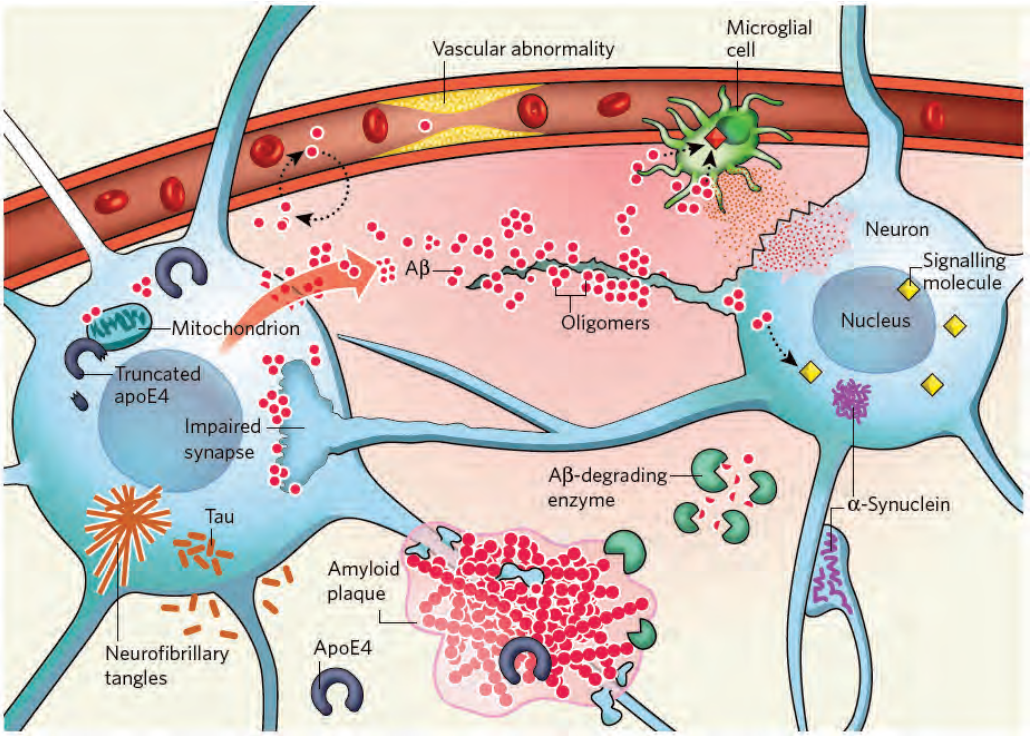


Figure 1. Some key players in the pathogenesis of Alzheimer's disease. Aβ: amyloid β, ApoE4 : apolipoprotein E ε4. Reproduced with permission from L Mucke, *Nature* 461; 15, 2009.

The dominating hypothesis for the cause of AD is the amyloid cascade hypothesis.[23, 24] According to this hypothesis, abnormal metabolism of the amyloid precursor protein (APP) and the subsequent accumulation of toxic Aβ peptides are key events in AD pathology. Accumulated Aβ is thought to lead to neuronal degeneration and functional loss via toxic effects and down-stream events

including tangle formation, oxidative stress and chronic inflammation. Support for a central role of Aβ in AD pathology includes the finding that the mutations implicated in familial AD are present in genes related to Aβ production. So far, several mutations have been found in APP[25-28] and in the genes encoding for the enzymatic centre of the γ-secretase complex, presenilin 1 and presenilin 2.[29, 30] Although there is a substantial body of evidence in support of the amyloid hypothesis, the triggers of amyloid aggregation in sporadic cases of AD are still not understood, and most importantly, there are no known means to alter or manipulate the amyloid cascade for a more favorable prognosis.

INFLAMMATION AND AD

Inflammation is the body's response to infections and tissue injury.[31] The inflammatory response is orchestrated by the cells of the immune system, both from the "adaptive" branch (including T- and B-cells with the capacity to induce long-term memory of encountered pathogens, "immunisation") and the "innate" branch (including monocytes, macrophages, dendritic cells, and mast cells etc., that are targeted against common pathogen antigens). Inflammation was first implicated in the development of AD in the beginning of the 90's when two key discoveries were made. The first discovery was that immune competent cells (activated microglia and astrocytes) and inflammatory proteins (e.g. cytokines and complement) are found in the vicinity of the amyloid plaques and the neurofibrillary tangles.[32] Many of the earliest results were at first dismissed as inaccurate given the perception of the brain as an "immune privileged" organ, i.e. an organ that does not elicit inflammation in response to antigens or damage. However, there is now an abundant literature on the presence of acute phase proteins in amyloid plaques, activated microglial cells that stain for inflammatory cytokines, and components of the complement system in brain tissue of AD patients.[33, 34]

The second finding was that arthritis patients and other patient groups with a high consumption of non-steroidal anti-inflammatory drugs (NSAID) had a lower proportion of individuals affected with AD. There are now numerous observational studies that have investigated the association between NSAIDs and AD, the findings of which have been summarized in several reviews.[35-38] In conclusion, these studies indicate that there is a dose-response relationship between NSAID use and the relative risk of AD, with longer periods of use related to reduced relative risks of AD. Based on these studies, the RR of AD appears to be 25-50% lower in groups of individuals with long-term (2 years or more)

NSAID use. The reduction in risk also appears to be restricted to AD; no protective effect against vascular dementia has been noted. The hypothesis is that the inflammatory response to the accumulating amyloid and tau deposits worsens the pathological process and that NSAIDs may alleviate the process by inhibiting the inflammatory response and/or inhibiting glutamate excitotoxicity.[39] Recent findings also suggest that it might not be the anti-inflammatory properties (cyclooxygenase inhibition) that mediates the protective effect but that some NSAID compounds have effects on amyloid processing.[40] Triggered by the findings in observational studies, several clinical trials have investigated the possibility of treating AD with NSAIDs, but with no success.[41-43] Whether these null findings are due to the wrong choice of investigated chemical compound or whether NSAIDs only possess the ability to delay AD onset rather than to mend what is already broken, remains to be proven.[44]

Since the initial discovery of a potential inflammatory ingredient to the AD cocktail, studies have diversified to look at a multitude of inflammation-associated risk factors for cognitive function, cognitive decline, AD, dementia and progression in dementia; including circulating inflammatory markers,[45-59] CSF markers of inflammation,[60-62] genetic sequence variation in immune-related genes,[63-71] and proxies of inflammatory load (e.g. gingivitis).[72] The mechanisms by which peripheral inflammation could affect AD development are not known but are hypothesized to be either as contributors to neuronal degeneration and/or as factors that lower the clinical threshold for dementia. As previously mentioned, the brain has traditionally been viewed as an immune-privileged organ since the normal immunological surveillance of the immune system does not pass over to the brain. However, it is now clear that blood borne cytokines can cross the blood-brain barrier (BBB) at specific sites and when the BBB is damaged[73-75] and that there are definite neuro-immune interations.[76, 77] In regards to circulating markers of inflammation, some studies have interpreted elevated blood levels as a spill-over from ongoing inflammation in the brain. Nevertheless, most researchers dismiss this possibility given that the concentration of inflammatory proteins in the brain is low whereas there are numerous other sources of low-grade inflammation that are often found in elderly populations[78] such as fat tissue,[79] smoking, subclinical infections and chronic disease.

An interesting development during the last decade is that traditional risk factors of cardiovascular disease also have been linked to AD. High blood pressure (BP), elevated cholesterol levels, obesity,

smoking, diabetes, and atherosclerosis have all been associated with AD. Similarly, exercise has been associated with a reduced risk.[80] It is possible that low-grade systemic inflammation constitutes a common denominator in neurodegenerative and vascular diseases, possibly via detrimental effects on the vasculature, leading to a dysfunctional BBB and inflammatory stimuli of the brain.

There are multiple possible explanations for the relationship between elevated peripheral inflammation and cognitive impairment, AD or dementia. One possibility is that inflammation-associated genes are also related to dementia, an individual with a pro-inflammatory genotype would manifest pro-inflammation both in the periphery and in the brain.[81] Elevated peripheral inflammation could also affect brain inflammation by "priming" of neurons, i.e. making them more prone to a pro-inflammatory response in the presence of tissue damage.[82-84] In addition, chronic inflammation during development and childhood could negatively affect brain development and lower the "cognitive reserve".[85] Chronic peripheral inflammation could also lower the threshold for clinical symptoms of AD and dementia. Another possibility to consider is reverse causation, i.e. that inflammation is not a risk factor for AD/dementia but the disease process will affect levels of circulating inflammatory markers. There is also the possibility that these hypothetical pathways are intertwined and that e.g. amyloid deposits in the cerebrovascular wall will elicit a peripheral inflammatory response that will in turn enhance brain inflammation.

INFLAMMATION-ASSOCIATED RISK FACTORS

Below follows a short description of the inflammation-associated risk factors included in this thesis.

Atopy

Historically, asthma, rhinitis and eczema have been thought of as separate disease entities with common features. However, recent findings indicate that the similarities outnumber the differences and there is now an emerging view that these are variable manifestations of the same systemic disorder.[86, 87] Atopy is defined as a propensity to produce IgE antibodies against allergens and manifests clinically as asthma, dermatitis, rhinitis and/or conjunctivitis.[88, 89] It's not uncommon to suffer from more than one of the disorders, either simultaneously or at different points in life.[90] However, not all cases can be attributed to atopy. It is estimated that 10-45% of atopic cases have symptoms without any IgE production.[91] On a population level, non-allergic atopic manifestations

have a higher age of onset, have more severe symptoms and a female pre-dominance compared to allergic forms of the same disorders.[91, 92] Although allergic atopic inflammation differs from a non-allergic atopic inflammation with regards to immune cells involved, cytokine patterns and antibody production,[93] there is no scientific consensus as to whether these differences are negligible or important markers of two truly distinct forms of disease. For the biologic rationale of this study it is important to note that besides the localized inflammation, these diseases are associated with multiple systemic manifestations and also have neuromodulatory properties. [94-101]

Because of the immunological and inflammatory properties of atopic disorders, asthma, rhinitis and eczema have received an increasing amount of attention during the last years as a potential risk factor for atherosclerosis.[102-105] Findings indicate that common allergic diseases could enhance the risk of CVD through a direct effect on the atherosclerotic process, although studies are far from conclusive.[106, 107] To my knowledge, no studies to date have investigated the association between atopic disorders and dementia in a longitudinal setting. Also, given that both AD and atopy are highly heritable diseases a genetic link between the two diseases is not biologically unlikely. The twin methodology provides an excellent setting for investigating the presence of such shared genetics.

Atherosclerosis and cardiovascular disease

Cardiovascular disease (CVD) and risk factors for CVD have been linked to cognitive impairment and dementia.[108-112] CVD is a well established risk factor for vascular dementia (VaD) but the association with AD remains unclear. The main underlying cause of CVD is atherosclerosis.[113] Atherosclerosis is an inflammatory disease of the blood vessels that eventually can lead to vessel rupture or thrombosis. If this occurs in the cerebral blood vessels (stroke) or the coronary arteries (myocardial infarction) this can be deadly. It has been hypothesized that atherosclerosis-induced brain hypoperfusion, oxidative stress and/or inflammation could contribute directly to the development of the neuropathology in AD.[114] However, CVD and AD are both prevalent diseases in elderly people and co-exist in a large proportion of individuals with late-onset dementia and recent data show that late-life dementia often represents a mix of AD and vascular pathology.[115, 116] Accordingly, many elderly persons with clinical dementia have both AD-type and vascular brain lesions.[117, 118] An alternative explanation is thus that CVD is simply a comorbid process that increases the likelihood of a dementia diagnosis in patients with sub-clinical AD pathology.[119]

Another link between CVD and AD has been proposed to be the apolipoprotein E ε4 allele (APOE4). APOE4 is the most well-established genetic risk factor for sporadic AD (with a 3-fold elevated risk for carriers of one allele and up to a 15-fold increased risk for carriers of two alleles compared to the reference level ε3/ε3) and has also been linked to CVD, although this association has recently come in to question. However, the role of APOE4 in the association between CVD and AD is unclear. Studies indicate that APOE contributes to AD pathology through direct effects on amyloid beta processing and neurotoxicity and not through enhanced atherosclerosis and cardiovascular disease. Some studies have also found that there is an interaction between APOE4 and CVD on AD risk while others have not. The Swedish Twin Registry in combination with collected information on APOE genotype provides an excellent setting for investigating the association between CVD and AD while adjusting for underlying genetic factors and testing for interactions with APOE4.

Anti-phosphorylcholine

IgM antibodies against phosphorylcholine (anti-PC) are thought to be naturally occurring antibodies with vascular protective and anti-inflammatory effects. Levels of anti-PC have been associated with life-style factors and proposed to provide a link between a "westernized" life-style and atherosclerosis and CVD. This interpretation was spurred by the finding that hunter-gatherer societies in New Guinea, where CVD is presumably low, have high levels of anti-PC compared to societies with western life-styles.[120]

Oxidized lipids, like oxidized low density lipoprotein (oxLDL), are a hallmark of atherosclerosis. OxLDL is pro-inflammatory and can activate monocytes, endothelial cells, B- and T-cells through phospholipids like lysophosphatidylcholine and/or platelet activating factor (PAF)-like lipids.[121, 122] Phosphorylcholine (PC) is a prerequisite for the binding of inflammatory phospholipids to the PAF-receptor, causing pro-inflammatory effects.[123] Anti-PC thus exerts anti-inflammatory properties by inhibiting endothelial activation induced by inflammatory PAF-like and PC-exposing phospholipids, suggesting that low levels of anti-PC could predispose to chronic inflammation. It is thus possible that low anti-PC could promote AD by decreased inhibition of inflammatory phospholipids. Indeed, phospholipase A(2) (PLA[2]) enzyme has been linked to memory impairment and neurodegeneration in AD.[124] Besides inflammation, it is interesting to note that a microglia receptor for Aβ (which

induces H_2O_2 production)[125] also binds PC-containing oxLDL.[126] Anti-PC is thus an interesting marker in relation to AD since it appears to have effects on both lipid metabolism and inflammation.

C-Reactive Protein and Interleukin-6

Interleukin-6 (IL6) and C-reactive protein (CRP) are both inflammatory proteins that are secreted upon infection or tissue injury. IL6 is a key regulatory cytokine produced by a variety of cell types including leucocytes, adipocytes and cells of the nervous system,[33, 127] and is also the main regulator of the acute-phase reactant CRP.[31] CRP is secreted by the liver and levels increase dramatically upon infection. CRP is therefore measured in clinical practice as an indicator of current infection. CRP and IL6 have been found in association with senile plaques and neurofibrillary tangles and to be elevated in temporal cortex in subjects with AD.[128-130] However, studies of circulating inflammatory markers and genetic variation in inflammatory genes in relation to AD are inconclusive.[131, 132] Circulating CRP have been shown to predict cardiovascular events in multiple prospective studies[133-135] but there have been few similar attempts to evaluate associations of inflammatory markers with incident AD and dementia.

Twin studies have shown that over 50% of the genetic variation in cytokines is genetically determined[136, 137] which provides strong evidence for a genetic basis of inflammatory mediated diseases. It is thus possible that the discrepant findings between circulating CRP/IL6 levels and AD are influenced by genes. The twin population of this study provides an outstanding framework for testing if genes influence the association between circulating CRP/IL6 levels and the risk of developing dementia.

THE TWIN METHOD

Twins provide an excellent framework to disentangle genetic and environmental effect on a trait or a disease.[138] The twin method takes advantage of two factors, the first relating to genes and the second to environment (with environment defined as all non-genetic factors). Monozygous (MZ) twins (two individuals who come from the same fertilized egg) share all of their segregating genes whereas dizygous twins (DZ) (who stem from two different fertilized eggs as do siblings in general) share on average half of their segregating genes. Thus, MZ pairs and DZ pairs differ on within-pair genetic similarity whereas they are assumed to share the early environment to the same extent. The

classical twin method thus takes advantage of the assumption that MZ pairs will differ from DZ pairs only on the degree of genetic correlation. Simplified, greater resemblance between two MZ twins (within a pair) as compared with two DZ twins (within a pair) indicates genetic influence on a trait or, in other words, if MZ twins are more likely to be similar to their twin partner on a trait (e.g. height) than are DZ twin pairs, it points toward a role of genes for that specific trait.

This thesis includes so called “co-twin control” analyses. Co-twin control analyses are identical to matched case-control analyses with each strata containing one twin pair. Co-twin control analyses are based on twin pairs who are discordant for both the disease and the outcome. The non-diseased twin partner is used as a control do the diseased proband. Twin pairs are not only matched for age but also for un-measured familial factors (i.e. early rearing environment and genetic architecture). If relative risks estimated in co-twin control analysis are substantially different from relative risks estimated in the full cohort, this indicates that familial factors are confounding the association between the exposure and the outcome.

3 AIMS

The overall objective of this thesis was to study different aspects of ***peripheral inflammation as potential risk factors for dementia in general and Alzheimer's disease in particular***. To address the overall aim, the four studies included in the thesis had the following specific aims:

- To study the effect of atopic diseases on the risk of developing AD and dementia.
- To investigate the effect of cardiovascular disease (other than stroke) on the risk of developing AD and dementia.
- To study the association of circulating levels of antibodies against phosphorylcholine with prevalent and incident AD and dementia.
- To study the associations of gene sequence variation and serum levels of C-reactive protein and interleukin-6 with AD and dementia.

4 MATERIALS AND METHODS

STUDY SETTING

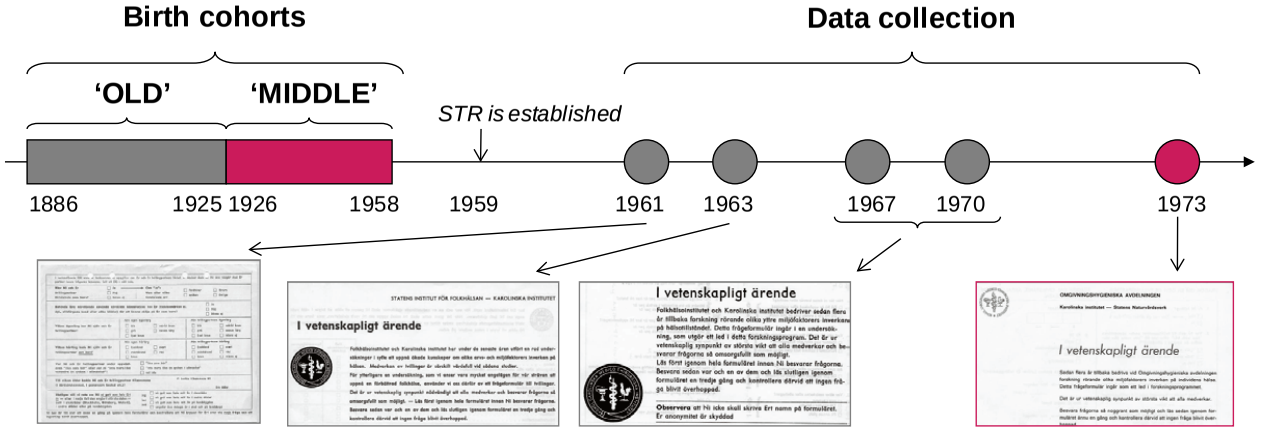
The study participants included in the work of this thesis are Swedish twins born 1886 through 1958 and included in the population-based Swedish Twin Registry (STR). Study IV also includes a non-twin sample of Swedish late-onset Alzheimer's disease cases and non-demented controls (LOAD CC sample).

DATA SOURCES

The Swedish Twin Registry

The Swedish Twin registry (STR) is a population-based and nation-wide registry that covers 96% of all twins born in Sweden.[139] Today the STR includes approximately 170,000 twins in 85,000 pairs, in principle all twins born from 1886 to 2000.[140] The STR, administered at the Karolinska Institutet, was initiated in the late 1950's with the main objective to study morbidity and mortality associated with smoking while controlling for familial factors that could predispose to disease. [141]

Compilation of the registry is described in Figure 2. The assembly of the STR was initiated by contacting all parishes (*sv. församlingar*) in Sweden to obtain information on multiple births between 1886 and 1925.[139] This birth cohort is referred to as the "old" cohort of the STR and comprises approximately 10,000 twin pairs. In 1970, the compilation of a new birth cohort covering the years 1926-1967, referred to as the "middle" cohort of the STR, was initiated by searching nationalized birth registrations and a register consisting of 50,000 twin births was established. The STR now includes information on twin births until 2000. Birth data is available on all individuals in the registry (including unlike-sex pairs) whereas questionnaire/study information has been collected only on selected subsamples. Given that this thesis is based solely on twins born until 1958, no further description of the younger birth cohorts will be given.



Q61	*Q63*	*Q67/Q70*	*Q73*
Twin similarity			Twin similarity
Marital status	Family status	Family status	Family status
Smoking		Smoking, alcohol, diet	Tobacco, alcohol, diet
Urban/rural	Urban/rural	Urban/rural	Urban/rural
	Education, occupation	Occupation	Education, occupation
	Height, weight	Height, weight	Height, weight
	CVD & respiratory sympt.	CVD & respiratory sympt.	CVD & respiratory sympt.
	Selected diseases	Selected diseases	
		Physical activity	Physical activity
		Psychosocial	Psychosocial
		Stress	Environmental stressors
			Selected medications
	Accidents		
	Dental status		

Figure 2. Compilation of the Swedish Twin Registry. Circles indicate questionnaire studies. Grey fill refers to the old STR cohort and plum fill refers to the middle STR cohort.

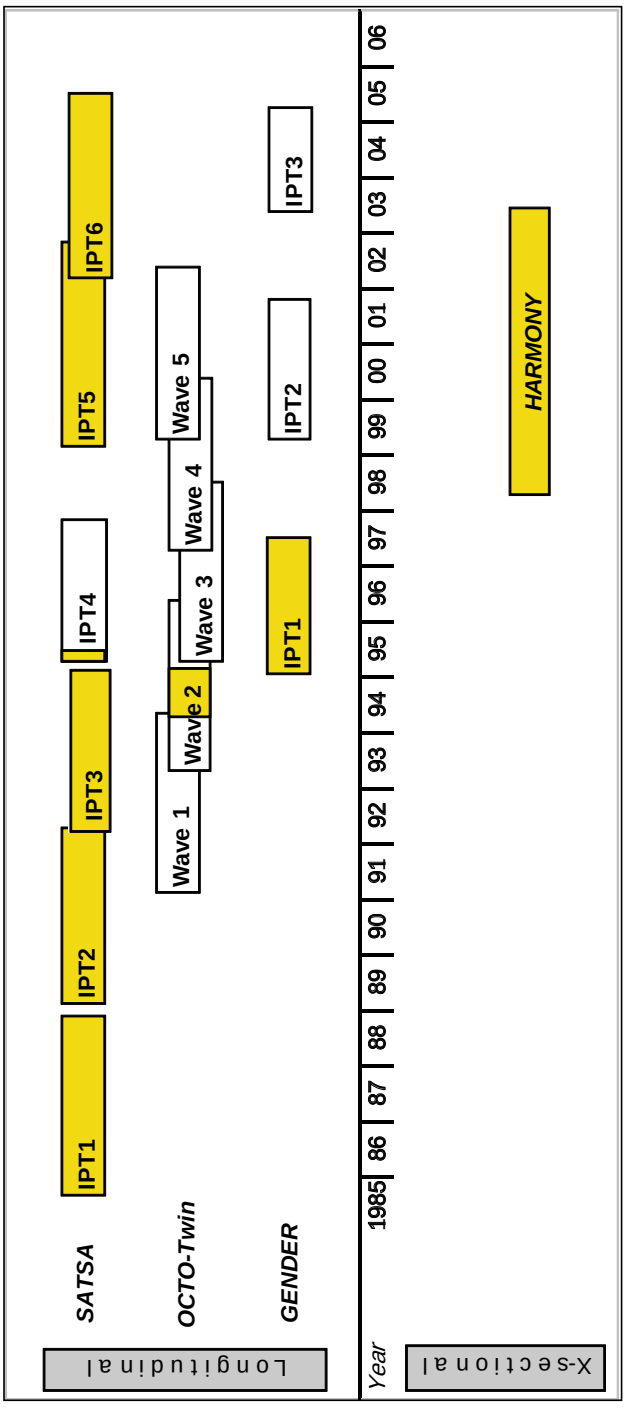


Figure 3. Baseline and follow-up sessions in SATSA, OCTO-Twin, and GENDER

Filled boxes indicate sessions including blood sampling. IPT: In-person testing.

Questionnaires

In the early 1960's, a paper questionnaire (Q61) was sent to all like-sexed twin pairs of the old cohort where both were alive and living in Sweden, inquiring basic demographic and smoking information (Figure 2).[142] Additional questionnaires were sent out (to the responders of the initial questionnaire) in 1963 (Q63) and 1967 (Q67) (with a follow-up to non-responders in 1970 [Q70]) querying demographic, medical and life-style factors and with special attention to symptoms of cardiovascular and respiratory disease. From Q61 and Q63, information was only stored from those pairs where both twins had responded. Although the response rate on an individual level was 90.9% and 91.7% for Q61 and Q63, respectively, the corresponding twin pair response rate is approximately 85% for both questionnaires.[138] The response rate for Q67 was 82%.[143] In 1973, a sub-set of the middle cohort (those born 1926-1958) was approached with a similar questionnaire to Q67.[141] Q73 was sent to the almost 39,000 individuals and responses were received from approximately 32,400 (corresponding to a response rate of 83%) and included almost 14,000 complete pairs.

Zygosity has been assigned according to a set of questions about intra-pair similarity in childhood queried in Q61 and Q73. This method has been validated against DNA as having at least 98% accuracy.[139]

SATSA

The Swedish Adoption Twin Study of Aging (SATSA) is an ongoing longitudinal study, launched in 1984, with the primary aim of quantifying the relative contribution of genetic and environmental factors on different measures of aging and cognitive abilities.[144-146] During the compilation of the old and middle cohorts of the STR (i.e. Q61 and Q73) it was noted that a discernable number of twin pairs reported having been separated from each other in early childhood and reared apart (mostly due to death of a parent or economic hardship). The adoption-twin study design takes advantage of the fact that twins are genetically related but reared in different environments and thus provides a framework for separating genetic from environmental influences on a trait.

The "core" population for SATSA consists of the 961 like-sexed twin pairs (1,922 individuals) where one or both members reported (in Q61 or Q73) that they had been separated before the age of

eleven.[144] A control sample of 627 intact twin pairs reared together (TRT) and 197 single-surviving TRT individuals (824 "source" pairs and 1,451 individuals) were matched on gender, age and county of birth.[145] In 1984, both members of 591 twin pairs reared apart (TRA) and one member of 221 single TRA individuals were alive (i.e. corresponding to 812 "source" pairs and 1,403 individuals). A questionnaire (Q1) was sent to all 2,854 individuals alive in July 1984; 2,018 responded (758 complete pairs).

There are two parts to the data collection in SATSA. One part consists of mailed questionnaires and the second part consists of in-person testing (IPT) on a subset of the twins on a 3-year rolling basis (with a hiatus of seven years between IPT3 and IPT5) (Figure 3). IPT involved an interview, administration of cognitive tests, a health examination, and fasting blood sampling. In order to be contacted for IPT, both members of the pair had to answer to Q1 and be 50 years of age or older. In 1986, 548 of the 758 complete pairs who responded to Q1, fulfilled the criteria and were invited to participate. IPT1 was conducted between 1986 and 1988. All individuals who participated in IPT1 (regardless of the survival status of their twin partner) were contacted for testing in IPT2. In addition, the subsample of pairs responding to Q1 who turned 50 between 1986 and 1989 were also contacted for IPT2. All twins who had participated in any IPT plus those who had answered to Q1 and turned 50 after last IPT were contacted for subsequent IPTs. IPT4 was reduced to a telephone-based brief cognitive screening test due to funding considerations. In summary, 645 individuals were included in IPT1, 595 in IPT2, 569 in IPT3 and 545 in IPT5.[146]

In SATSA, cognitive function was evaluated with a cognitive battery including the MMSE, or if by-telephone the TELE (to questionnaire non-responders). Twins who screened positive for suspicion of dementia (and their twin partners) were further evaluated through physical and neurological examinations, informant interviews, reviews of medical records, laboratory tests and neuroimaging (in large following the CERAD protocol). Mean age at baseline examination was 65.0 years (standard deviation [SD] 10.0). All together, there are 1,089 unique individuals with information on cognitive status. Final diagnoses of dementia were set at a multidisciplinary consensus conference according to the DSM-III-R criteria and differentially diagnoses as AD (based on the NINCDS-ADRDA criteria), VaD (based on the NINDS-AIREN criteria), mixed AD and VaD, other specified dementia or unspecified dementia. As per august 2008, there are 214 individuals with a diagnosis of dementia (129 AD, 37 VaD, 11 mixed AD/VaD, 24 dementia not otherwise specified [NUD], 2 dementia in PD,

and 11 secondary dementias) and 78 individuals diagnosed with questionable dementia. Of the 214 persons with dementia, 139 (65.0%) were diagnosed at the baseline examination and 75 (35.0%) were diagnosed at follow-up. The high proportion of dementia cases diagnosed at baseline is due to the pursuit of non-responders. Non-responders to Q1 who were above 55 were contacted for dementia assessment and several of the elderly non-responders were diagnosed with dementia.

OCTO-Twin

The Origins of Variance in the Old-Old: Octogenarian Twins (OCTO-Twin) study is a longitudinal study of twin pairs above 80 years of age.[147] Like-sexed twin pairs alive and 80 years or older in 1991-1993 (and not already included in SATSA) were identified in the STR.[148] Of the 947 pairs who fulfilled the criteria, 737 pairs were selected to be contacted (the restricted number was a constraint imposed by funding). One or both twins of 188 pairs had died in the time that passed from the selection to the contacting of the twins and in another 198 pairs one or both twins declined to participate. Overall the response rate was 86%.[149] Thus, 351 complete pairs (702 individuals) remained and were invited to take part in five in-person testing (IPT) sessions of health and cognitive function on a two-year rolling schedule (Figure 3). Average age at baseline examination was 83.6 years (SD 3.2). Participants were tested individually in their place of residence by a licensed nurse and members in a pair were tested by different nurses. In 111 of the 351 pairs, one or both twins could not complete the testing session; in 88 cases this was due to suspected dementia and they were diagnosed at the consensus conference. Of the 702 individuals assessed in IPT1, 535 were alive and willing to provide a (non-fasting) blood sample in 1994.[150, 151]

The dementia ascertainment procedure in OCTO-Twin followed the same protocol as for SATSA (see above). There are 224 individuals with a diagnosis of dementia (127 AD, 56 VaD, 2 mixed AD/VaD, 33 NUD, 1 dementia in PD, 5 secondary dementias). An additional 17 individuals were diagnosed with questionable dementia. Of the dementia diagnosi, 104 (46.4%) were set at the baseline examination, 120 (53.6%) were given at follow-ups. The relatively low number of dementia cases diagnosed at baseline (given the age profile of the study) is probably due to having no additional follow-ups of non-responders.

GENDER

The Aging in Women and Men (GENDER) study is a longitudinal study of unlike-sexed pairs born 1906-1925.[152] A total of 1,699 pairs (3,398 individuals), who were alive in 1995, were identified and sent a survey. Of these, 1,843 individuals returned the survey (54% response rate). The responders consisted of 605 complete brother-sister pairs (N=1,210). Of the responders, a sub-sample consisting of 249 twin pairs (N=498) born 1916 through 1925 agreed to participate in IPT starting in 1995 and including an interview, administration of cognitive tests, health examination, drug registration and fasting blood sampling.[153, 154] Two follow-up IPTs were conducted on a four year rolling schedule (Figure 3). Average age at baseline was 74.5 years (SD 2.6).

Dementia ascertainment was based on cognitive testing (including the MMSE), medical record reviews, interviews, fasting blood sampling and nurse's evaluation on the Berger scale. Final diagnoses of dementia (including differential diagnosis) were set at a multidisciplinary consensus conference according to the DSM-IV criteria. There were in total 87 individuals diagnosed with dementia (31 AD, 34 VaD, 1 LBD, 13 NUD, 8 secondary), 19 (21.8%) with a baseline diagnosis and 68 (78.2%) with a diagnosis during follow-up.

HARMONY

The Study of Dementia in Swedish Twins (HARMONY) is a cross-sectional study started in 1998 with the aim of complete ascertainment of dementia in all twins 65 years or older (i.e. born <=1935).[4] The ascertainment procedure included two phases; first a cognitive screening using a structured telephone interview and second, a clinical dementia evaluation of all twins who screened positive for cognitive dysfunction, their twin partners and a sample of 35 twin pairs who screened negative for cognitive dysfunction. All 20,269 twins who were alive in 1998 (regardless of pair status) and fulfilled the age criteria were invited to participate in the structured telephone interview.

Cognitive screening entailed the TELE and the BDRS (in case of an informant interview). All twins suspicious of dementia, their twin partners and a sample of 35 twin pairs negative for suspicion of dementia were referred for clinical dementia evaluation (in general following the CERAD protocol) and included physical and neurological examination; a complete medical history based on medical record review and informant interview, including use of prescription and non-prescription

medications, onset and sequence of memory and cognitive symptoms; a neuropsychological assessment; collection of blood for laboratory tests; and referral for neuroimaging.

In total, 14,435 participants were reached for interview either in person or by proxy (N=712) at an average age of 73.9 years (SD 6.8). Among those who finished the telephone screening, 11.5% were positive for cognitive dysfunction. A total of 2,139 twins were invited to the clinical phase. The final sample consists of 1,557 individuals with a cognitive evaluation of whom 620 have a diagnosis of dementia. Of these individuals, 86 belong to the SATSA population, 81 to the OCTO-Twin population, and 16 to the GENDER population, leaving 437 dementia cases unique to HARMONY (260 AD, 6 AD/FTD, 3 AD/LBD, 1 AD/PD, 4 FTD, 2 LBD, 91 VaD, 15 mixed AD/VaD, 28 NUD, 8 dementia in PD, 19 secondary dementias).

National Disease Registers

The STR is regularly updated against several national disease registers. Linking registries together is possible due to the Swedish personal identity number (PIN) (*sv. personnummer*).[155] The PIN consists of a unique ten digit number assigned to all individuals since 1947 who have resided on a permanent basis in Sweden. Statistics Sweden established the Total Population Register when the local population registers were computerized in 1967. In 1991, the responsibility for the local population registers was moved from the local parishes to the local tax offices and since then the National Tax Board is responsible for the Swedish PIN. The first six digits in the PIN correspond to the date of birth in YYMMDD format. The following two digits are unique to each individual; the pen-ultimate digit indicates the sex of the person with even numbers for women and uneven for men. The last digit is a control number. Below follows a description of the national disease registers linked to the STR and included in this thesis.

National Patient Register

The National Patient Register (NPR) (sometimes referred to as the Hospital Discharge Register) is administered by the Swedish National Board of Health and Welfare (SNBHW) (*sv. Socialstyrelsen*) and includes information about all in-patient care (i.e. over-night hospitalizations) at public hospitals in Sweden.[156] The NPR was initiated in 1964 and reached nationwide coverage in 1987 (Figure 4). A register of psychiatric care was introduced in 1973 on a national basis and this register is now included in the NPR. Approximately 99% of all public hospitalizations are included in the register

and, given that Sweden has had no or very few private providers of inpatient care, can therefore be considered to be population-based.

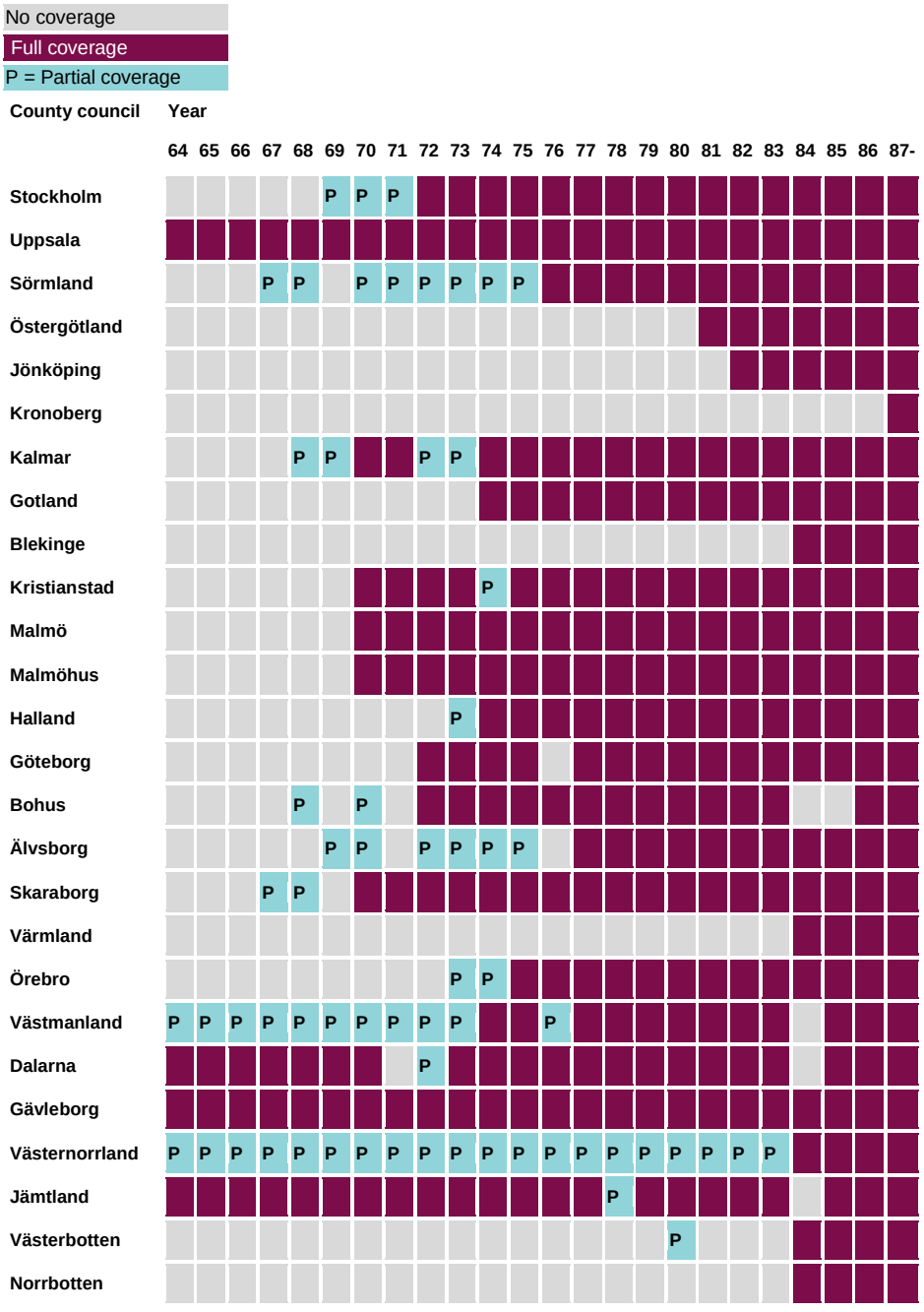


Figure 4. Coverage in the Swedish National Patient Register by year and county council.

Each record in the NPR corresponds to one hospital admission and contains the dates of admission and discharge, with diagnoses coded according to International Classification of Diseases (ICD), Seventh Revision (ICD-7, 1964–1968); Eighth Revision (ICD-8, 1969–1986); Ninth Revision (ICD-9, 1987–1996); and Tenth Revision (ICD-10, 1997–). The primary and up to six (1964-1996) or eight (1997-) additional diagnoses are recorded for each hospitalization along with administrative data about the patient and the treating hospital/clinic.

Causes of Death Register

The Causes of Death Register (CDR) includes all deceased individuals who in the year of their death were registered as residents in Sweden, irrespective of whether the death occurred in Sweden or abroad.[157] The register does not include stillborn, individuals who died during a temporary stay in Sweden or individuals applying for asylum but without a permanent residency in Sweden. Emigrated Swedes no longer registered in Sweden are also not included. The CDR includes nation-wide data since 1961 and is updated on a yearly basis. According to the SNBHW, the CDR fails to log a cause of death for less than 0.5% of all deceased individuals in a year.

Underlying and contributory causes of death are registered in the CDR according to the ICD system (*see National Patient Register*). The number of causes of death that can be entered in the CDR has increased form one underlying cause and five contributing causes for the period 1961-1986 to seven contributing causes for 1987-1996 and from 1997 and onwards it is possible to give up to 19 contributing causes of death.

Swedish non-twin case-control sample (LOAD CC)

The Swedish non-twin case–control sample (included in Study IV) consists of 896 late-onset Alzheimer's disease (LOAD) patients and 248 controls. These individuals were recruited from three prospective longitudinal studies of patients with dementia from Mölndal, Piteå, and Malmö, Sweden. In the AD cohort, 803 had a clinical and 93 a neuropathological diagnosis. All clinically diagnosed AD patients underwent a thorough investigation, which included a medical history, physical, neurological and psychiatric examination, screening laboratory tests, ECG, X-ray of the chest, EEG, and computerized tomography (CT) of the brain. MMSE was administered by a trained nurse and recorded in journals. When the study was completed and AD cases identified, journals

were reviewed for the latest MMSE score. Clinical AD diagnoses were made according to the NINCDS-ADRDA criteria. All neuropathologically diagnosed AD patients met the neuropathological CERAD criteria for definitive AD. Among controls, 140 were healthy volunteers without history, symptoms or signs of psychiatric or neurological disease, malignant disease, or systemic disorders. Cognitive status was examined using MMSE, and individuals with scores below 28 were not included as controls. There were 108 controls consisting of patients who had died from cardiac disease or malignant disease. Their medical records revealed no history of dementia or other psychiatric or neurological diseases. Post mortem examination revealed no macroscopic infarcts. All autopsy individuals (AD and control) were matched by age at death and all clinically diagnosed AD cases and healthy volunteers were matched by age at onset/age at exam, respectively.

DEMENTIA and AD ASSESSMENT

The gold standard for AD diagnosis is based on post-mortem neuropathology. In the (ante-mortem) clinical and research settings, dementia is assessed by conducting a physical examination, a medical history review, laboratory tests, neuroimaging, and neuropsychological testing. Neuroimaging is not a part of the standard work-up but can help to rule out tumors and infarcts and to differentiate AD from other forms of dementia. In epidemiological studies it is common to use a two-stage design, with brief tests (with high sensitivity) that can be administered in-person or over the telephone to do a primary assessment of cognitive function. Individuals who score below a pre-determined threshold for suspicion of dementia are then invited to a complete diagnostic work-up (with high specificity). Below follows a summary of the procedures used for cognitive screening and dementia diagnosis of relevance to this thesis.

Screening for cognitive dysfunction

MMSE. The Mini-Mental State Examination (MMSE)[158] is one of the most frequently used tests for neuropsychological assessment in clinical practice and research. The test is used both to screen for suspicion of dementia and as a tool to monitor longitudinal change in cognitive function. The test is administered as a face-to-face interview with questions aimed to assess orientation, registration, attention and calculation, recall, and language. Scores of 24 or above (out of 30) are generally considered as normal, 19-23 as mild cognitive dysfunction, 13-18 as moderate cognitive dysfunction, and 12 or less as severe cognitive dysfunction. The MMSE has been shown to correlate with age and educational level[159] and therefore age- and education-adjusted cutoffs are often applied.[159] The

MMSE was used in SATSA, OCTO-Twin and GENDER as part of the screening for cognitive dysfunction.

TELE and BDRS. In epidemiological studies, face-to-face testing, such as the MMSE, can be too expensive and time-consuming. The TELE[160, 161] is a telephone assessment tool for dementia based on the 10-item Mental Status Questionnaire (MSQ) but supplemented by other cognitive items (counting backwards, recalling three words, and word similarities) and by questions about health and daily functioning. The TELE was used in SATSA to evaluate cognitive functioning in non-participants and in HARMONY as the primary cognitive screening tool. The TELE has a maximum score of 20 and usually applies a score of 15 or less as a cut-off for cognitive impairment.[161]

For HARMONY individuals who performed poorly on the TELE, an informant was interviewed with the Blessed Dementia Rating Scale (BDRS).[162] The BDRS evaluates the extent to which cognitive function interferes with daily functioning. It ranges from 0 to 28 and usually has a cutoff score of 1.5 to define cognitive dysfunction.[163] The results of the TELE and the BDRS were then transformed into an ordinal scale, with 0 as "cognitively intact", 1 as "minor errors", 2 as "poor performance", and 3 as "cognitive dysfunction". Individuals with a score of 3 were then referred for clinical dementia work-ups.

Berger scale. The Berger scale[164] measures social dependency on a six-point scale. The scale was used in GENDER as part of the screening for dementia.[153]

Dementia work-up and diagnosis

CERAD. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD)[165] represents a brief and reliable assessment for AD diagnosis in clinical and research settings. CERAD contains a clinical and a neuropsychological assessment battery aimed at discriminating AD cases from healthy controls (rather than to distinguish AD from other types of dementia). The clinical battery takes approximately 30-40 minutes to complete and contains semi-structured interviews of the subject and an informant, general physical, neurologic, and laboratory examinations, a drug inventory, a depression scale and a general medical history. The neuropsychological battery is designed to measure the principal cognitive manifestations of AD (memory, language, praxis, and general intellectual status) and includes the following tests: Verbal Fluency, Modified Boston Naming Test,

MMSE, Word list memory, Constructional Praxis, Word List recall, Word list recognition. The tests require approximately 20-30 minutes to be completed. Based on the data collected according to the CERAD protocol, AD diagnosis can be set according to the NINCDS-ADRDA criteria (see below). CERAD has also developed a standardized protocol for the neuropathologic evaluation of AD from autopsy brains.[166] The CERAD neuropathologic diagnosis of AD (not to be confused with the NINCDS-ADRDA clinical diagnosis of AD) is based on a semi-quantitative rating of atrophy of the ventricles, hippocampus and neocortex; number of senile plaques and neurofirbillary tangles in the neocortex; and evidence of cerebrovascular disease.

DSM. The Diagnostic and Statistical Manual of Mental Disorders (DSM) is published by the American Psychiatric Association and provides a classification of mental disorders.[167] The third revision (DSM-III) was published 1980 and was coordinated with the development of the ninth version of the International Classification of Diseases (ICD-9), published 1975. DSM-III was revised (DSM-III-R)[168] 1987 and the fourth edition (DSM-IV)[169], which is the edition currently in use, was published 1994. DSM-IV was developed in close proximity to ICD-10, which was published 1992. According to the DSM criteria, dementia is diagnosed when impairment is displayed in memory and executive (or other higher cortical) function and the loss of function represents a significant decline from a previous level and is sufficient to interfere with social or occupational activities. These criteria were used in SATSA, OCTO-Twin, GENDER and HARMONY for a diagnosis of dementia and thus form the basis of the clinical dementia diagnosis in Studies I-IV.

NINCDS-ADRDA.[170, 171] The National Institute of Neurological and Communicative Disorders and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) developed a set of criteria in 1984, for a clinical diagnosis of possible or probable AD and with histopathologic confirmation of brain tissue for a diagnosis of definite AD. The criteria are mainly used in research studies. According to the NINCDS-ADRDA criteria, a diagnosis of probable AD can be made with confidence if there is a typical insidious onset of dementia with progression and if there are no other systemic or brain diseases that could explain the cognitive deficits. A diagnosis of definite AD requires a clinical diagnosis of probable AD that is confirmed by histopathology. A diagnosis of possible AD is made in the presence of other significant diseases or if the clinical presentation is atypical.

NINDS-AIREN. The Neuroepidemiology Branch of the National Institute of Neurological Disorders and Stroke and the Association Internationale pour la Recherche et L'Enseignement en Neurosciences (NINDS-AIREN) have developed a set of criteria for the possible, probable or definite diagnosis of VaD.[172] A clinical diagnosis of probable VaD requires the presence of both dementia (not caused by other known factors) and cerebrovascular disease, and a relationship between the two (e.g. onset of dementia within 3 months of a recognized stroke, abrupt deterioration in cognitive function, or stepwise progression of cognitive deficits). A clinical diagnosis of possible VaD is given when brain imaging is missing to corroborate the clinical symptoms or if the course is atypical. A diagnosis of definite VaD can be given if the clinical criteria for probable VaD can be verified with biopsy or autopsy data. The presence of neurofibrillary tangles or neuritic plaques exceeding that expected for age prohibits a diagnosis of definite VaD. Mixed AD and VaD or "AD with CVD" is used for patients who fulfill clinical criteria for possible AD and who also present clinical or brain imaging evidence of relevant CVD.

ICD. The International Classification of Diseases (ICD) is the international standard diagnostic classification for general epidemiological and many health management purposes. The ICD has been the responsibility of the World Health Organisation (WHO) since its creation in 1948 when the sixth revision was published. The 8th revision was published 1969 and dementia was defined as 'a syndrome of chronic or progressive nature' characterized by 'impairment of orientation, memory, comprehension, calculation, learning capacity, and judgment'. In addition, 'there may also be shallowness or liability of affect, or a more persistent disturbance of mood, lowering of ethical standards, exaggeration or emergence of certain personality traits, and diminished capacity for independent decision-making'.[173] ICD-9 was implemented in Sweden 1987 with a similar definition of dementia.[174] The ICD-10 criteria for dementia are 1) objective memory decline, 2) absence of clouding of consciousness 3) decline in emotional control, motivation, or change in social behavior, and 4) symptoms present for at least six months. The ICD-10 criteria for AD (code F00) are that the general criteria for dementia have to be met and that there is no evidence from the history, physical examination or special investigations for any other possible cause of dementia. The diagnosis can also be sub-categorized as early onset (F00.0), late onset (F00.1), atypical or mixed with VaD (F00.2) and unspecified AD (F00.9). For VaD (F01) the criteria are that the patient must display dementia, have an unequal distribution of deficits in higher cognitive functions (with some affected and others relatively spared), and that there is clinical evidence of focal brain damage and cerebrovascular

disease reasonably judged to be etiologically related to the dementia. VaD can be further divided into VaD with acute onset (F01.0), multi-infarct dementia (F01.1), subcortical VaD (F01.2), mixed cortical and subcortical VaD (F01.3), other VaD (F01.8), and unspecified VaD (F01.9).

BIOLOGICAL MATERIALS

Serum

Serum samples collected in SATSA, OCTO-Twin, GENDER, and HARMONY were used for analyzing anti-PC in Study III and CRP and IL6 in Study IV. Serum fractions were prepared from venous blood according to standard procedures and stored at -70°C until time of analysis (although subsets of the samples had been thawed previously for use in other studies).

Anti-PC. Measurement of serum levels of anti-PC IgM antibodies was performed with an enzyme linked immunoassay (ELISA; Athera CVDefineTM kit, Athera Biotechnologies AB, Stockholm, Sweden). The assay is based on PC covalently linked to bovine serum albumin (BSA) coated onto 96-well microtitre plates. The assay was carried out in accordance with the manufacturer's recommendations, essentially as described. The detection limit of Athera anti-PC and coefficients of variation have been previously published.[175, 176] Sera containing IgM anti-PC levels above the highest calibrator were further diluted and retested.

hsCRP and IL6. CRP levels were determined with a high sensitivity near infrared particle immunoassay rate (NIPIA rate) method (measurement interval 0.2-380 mg/L) using Beckman reagents on Synchron LX20 automated equipment (Beckman Coulter, Fullerton, CA USA). IL-6 levels were analyzed using the Quantikine high-sensitvity ELISA commercial kit by R&D systems (Minneapolis, MN USA) with a mean minimum detectable dose of 0.039 ng/L. The analyses were performed by trained personnel at the clinical chemistry laboratory at the Karolinska University hospital in Stockholm.

DNA

Venous blood samples for DNA extraction were collected by trained personnel. DNA was extracted according to standard protocols at the National Forensic Laboratory (GENDER and OCTO-Twin) or at the KI Biobank (SATSA & HARMONY). Due to the small amounts of DNA left for some of the study

participants all samples were subjected to whole genome amplification (WGA) using standard kits involving Phi29 DNA polymerase (Amersham, Arlington Heights, IL) prior to genotyping. Genotyping was then performed using the Illumina GoldenGate assay system on Illumina BeadStation 500GX equipment, currently housed and implemented at the Uppsala University SNP Technology Platform.

STATISTICAL ANALYSES

All statistical analysis were performed with the statistical software package SAS[177] versions 8.1 (Study I), 9.1 (Studies I, II, III, IV), and 9.2 (Studies II, III, IV).

Clustered data: accounting for correlations within twin pairs

In general, statistical measures of variability are calculated based on the assumption that the observations in the sample are random and independent from one another, i.e. the observations are uncorrelated and the value for one individual will not correlate with the value of another individual (averaged over the entire sample). In contrast, if a variable tends to be similar from one individual to another, the observations are no longer independent but correlated, or "clustered". Twin pairs can be considered as clusters containing two twin individuals and with the data within each cluster as correlated observations. In terms of correlation matrices, this can be described as the off-diagonal pairwise correlations being equal to zero for uncorrelated data and in the range from zero to one (one indicating perfect correlation) in the case of correlated data.

Standard errors are measures of the precision of the estimate and are related to sample size; the larger the sample size, the smaller the standard error. In the case of correlated observations, each observation cannot be viewed as contributing an equal and independent amount of information. Consequently, the effective "statistical" sample size will be smaller than if the observations were uncorrelated. The challenge with samples of correlated data is to extract the appropriate amount of statistical information from each observation.

GEE. The generalized estimating equations (GEE)[178-180] method offers one approach to the problem with correlated data. GEE is an iterative method that assigns different statistical weights to different clusters and then uses these weights to calculate the variance, the covariance and the correlation (based on the within-cluster similarity of the residuals). The estimated correlation from one cycle is then used to re-estimate the weights used in the next cycle to get a new estimate of the correlation.

This cyclic process is repeated until the estimates stabilize. The final estimate of the degree of correlation is then used to estimate a standard error that is a more accurate quantification of the precision of the parameter estimates (e.g. relative risk, odds ratio). Since correct specification of the mean and variance functions is sufficient for unbiased estimates, the GEE model does not fully specify the distribution of the responses in each cluster and is therefore called a quasi-likelihood model (as compared to the full likelihood model). With GEE, the computational complexity is a function of the largest cluster rather than the number of clusters, an advantage when there are many small clusters as is the case with twin data. In terms of this thesis, logistic regression analysis in Study I and Poisson regression analysis in Study II were fitted within a GEE framework (in SAS: PROC GENMOD, REPEATED SUBJECT=identification number unique to each twin pair, TYPE=exch) to adjust standard errors and measures of effect (OR and HR, respectively) for within twin pair correlations.

ALR. The GEE method considers the correlations among observations to be "nuisance" parameters and, although the pair-wise correlations are adjusted for, they are not actually estimated. In some instances, it can be of interest to estimate the pairwise correlations, e.g. for DZ and MZ pairs separately or for two longitudinal time points. Alternating Logistic Regression (ALR)[181] is an extension of GEE that parameterizes the association in terms of pair-wise odds ratios. The term "alternating" in ALR refers to the fact that the estimating algorithm alternates between estimating the log-odds of the outcome and the log-odds of the within twin pair association. ALR (in SAS: PROC GENMOD, REPEATED SUBJECT=identification number unique to each twin pair, LINK=logit, LOGOR=logorvar [cluster identification variable]) was used in Study IV in the analysis of the association between gene sequence variation and AD/dementia to account for the fact that MZ pairs share all their genes whereas DZ pairs only share on average half of their segregating genes.

Survival analysis

Methods of survival analysis are used for analyzing longitudinal time-to-event data. There are three key concepts to survival analysis: events, failure time and censoring time. The event is defined as the outcome of interest, e.g. dementia onset, and must have a known time of occurrence (e.g. date or age). The time from baseline to the occurrence of the event (the "failure") is referred to as failure time. When an event is not observed before the end of follow-up, the failure time is said to be right censored (given that we have no information about the event to the "right" of the end of follow-up)

and the follow-up period is then referred to as censoring time (instead of failure time). In survival analysis, the effect measure is the hazard ratio (HR). The HR is defined as the hazard in the exposed groups divided by the hazard in the unexposed groups. For all practical purposes, hazards can be thought of as incidence rates and thus the HR can be roughly interpreted as the incidence rate ratio.[182, 183]

Cox proportional hazards regression (Study I)

The Cox proportional hazards regression model[184] is a semi-parametric regression model for censored failure time data. This model assumes that covariates have multiplicative effects on the baseline hazard function. The "semi" in semi-parametric refers to the fact that the baseline hazard function does not have to be specified as is the case in fully parametric models. In Study I, relative risks were modeled as hazard ratios in a Cox proportional hazards regression model. Events were defined as hospitalization with dementia or death with dementia. Observations were censored due to death from other causes, loss to follow-up, or end of study. Adjusting for correlated data within the Cox model framework is not straightforward. However, this model was still our preferred choice since the alternative Poisson-model (see below) requires splitting the underlying time scale into intervals and there were too few events in the exposed groups to allow for reasonably small intervals. The validity of the model was assessed by comparing the Cox model to a Poisson model (both a naïve Poisson and a GEE Poisson model) and since there were no substantial differences in point estimates or standard errors between the models, the Cox model was considered valid. Statistical analyses were performed using the SAS 9.1 TPHREG procedure for the Cox model and the GENMOD procedure for the Poisson model.

Poisson regression (Study II)

Poisson regression belongs to the group of generalized linear models (GLM) and is often used when employing a person-time approach. The GLM generalizes linear regression by allowing the linear model to be related to the response variable via a link function.[185] Poisson regression models count data using the Poisson distribution and the link function is typically the logarithm, also called the "canonical" link. A Poisson regression model (PROC GENMOD with DIST=Poisson and OFFSET=natural logarithm of time at risk) was used to calculate HRs in Study II. Time at risk was calculated as years from baseline to the date of the first record of dementia, last date of follow-up, other causes of dementia (e.g. hydrocephalus), or end of study (while adjusting for age). The Lexis

macro[186] was used to split the underlying time scale. CVD was analyzed as a time-dependent variable, i.e. a person negative for CVD contributes time-at-risk to the CVD-negative group until the first record of CVD in the NPR, at which point that individual will start to contribute time-at-risk to the CVD-positive group. Testing the proportional hazards assumption revealed that the effect of CVD on dementia risk was affected by time since CVD and a second time scale (time since CVD) was therefore introduced. HRs were thus estimated by including (time since exposure*CVD) interaction terms (<= 3 years and >3 years since exposure) in the same model. We also adjusted for correlated twin data by fitting the Poisson model within a GEE framework.

Logistic regression

Logistic regression models are used to study effects of predictor variables on categorical outcomes (e.g. presence or absence of disease). The logistic regression model estimates odds ratios (OR) as a measure of effect.

Conditional logistic regression (Studies I-IV)

Conditional logistic regression (CLR) is used to analyze matched or stratified data of categorical outcomes. In this thesis, CLR was used in all four studies as the means of performing "co-twin control" analysis. Co-twin control analysis is the term used by twin researchers to refer to CLR matched on twin pair. In SAS, this was achieved with PROC LOGISTIC and with STRATA= identification number unique to each twin pair. CLR was also used in studies III and IV as the means of analyzing the nested case-control and prevalent case-control studies of serum levels of anti-PC, hsCRP and IL6 and the association with AD/dementia. This was also performed with PROC LOGISTIC but with STRATA= identification number unique to each matched set of one case to multiple controls.

Unconditional logistic regression (Studies III+IV)

In order to increase sample size and power in studies III and IV, the case-control matching was broken for some of the stratified analysis. In these instances, the matching variables (i.e. age at blood draw and sex) were included in the logistic regression model as explanatory variables.

Alternating logistic regression (Study IV)

See section on clustered data (above).

STUDY DESIGN and EXPOSURE ASSESSMENT

A summary of Studies I-IV is shown in Table 1.

Study I: Atopic disorders and Risk for Dementia

In Study I, we investigated the association of the atopic disorders asthma, eczema, and rhinitis with dementia and AD in a cohort of twins from like-sexed pairs born 1903-1936 and alive on January 1st 1974. The association with dementia was evaluated in a) a longitudinal setting with register-based dementia diagnosis collected in the NPR and CDR from January 1st 1974 to December 31st 2001 (N=22,188), and b) a cross-sectional setting with clinical dementia diagnosis from HARMONY (N=7,800). The inclusion criteria were defined so that the oldest twins would be younger than 65 years and unlikely to have dementia at time of exposure assessment and the youngest twins would reach 65 years by the end of follow-up.

Previous or current asthma, eczema and/or rhinitis were assessed through self-reporting in paper questionnaires (see above). Twins belonging to the old STR cohort were evaluated in Q63 and Q67 with specific questions regarding having or ever having had asthma, eczema or rhinitis. Twins belonging to the middle STR cohort were approached in Q73 with one combined question on asthma, eczema, rhinitis and false croup (the latter being a usually harmless viral upper respiratory tract infection that affects children below the age of six years). Data on asthma, eczema and rhinitis alone are thus only available on twins of the old STR cohort. Atopy was defined as any positive record of asthma, eczema, rhinitis (or false croup) in Q67 (or if missing, in Q63) and Q73. False croup was not of interest to this study but since it was included as a non-excludable alternative to the middle cohort, the variable was included to define atopy also in the old cohort.

Information on smoking and educational level was derived from the twins' self-reports in Q63, Q67 (Q70), and Q73. Data on non-fatal myocardial infarctions (MI) were extracted from the NPR. Non-fatal was defined as being alive at least one month after the MI. In the demented group, MIs were recorded until time of first dementia record. For equality reasons, MIs in the non-demented group were recorded until the mean age of dementia onset in the demented group, i.e. 79.5 years.

In co-twin control analysis, only like-sexed pairs where the partner of the proband was alive at the time the proband developed dementia were included. Concordant demented pairs were also included in the analysis, with the first twin to develop dementia as the case and the second twin to develop dementia as the control. No restrictions were imposed on the time span between the ages of onset of dementia within the pair.

Study II: CVD and Alzheimer's disease

In Study II, we investigated the association between cardiovascular diseases (other than stroke) and AD/dementia in a retrospective cohort of 2,214 twins included in any one of three longitudinal studies of aging in the STR with clinical dementia diagnosis: SATSA, OCTO-Twin and GENDER (henceforth referred to as the "clinical cohort"). We then replicated our analysis in a sample of 18,405 like-sexed twins born 1903-1936 with register-based dementia diagnosis from the NPR and CDR (henceforth referred to as the "register cohort").

Clinical cohort. Information on CVD was gathered through linkage to the NPR. All ICD codes (versions 8, 9 and 10) for hospital admissions due to angina pectoris (AP), MI, atherosclerosis, claudication, ischemic heart disease, and the surgical procedures coronary artery bypass graft (CABG) and percutaneous transluminal coronary angioplasty (PTCA) were considered. Being positive for CVD was defined as hospitalization with any of the above mentioned codes either as the primary or contributory cause of hospitalization. Two sub-categories were also formed, one for diagnosis of MI and another for diagnosis of AP.

Our baseline was defined as 1st January 1974. More than 50% of the Swedish counties were then covered by the NPR and included approximately 68% of the Swedish population (Figure 4). Another 10% of the population was at the time living in counties that were partially covered by the NPR. From the 2,287 twins with cognitive data in SATSA, OCTO-Twin, and GENDER we excluded 12 individuals due to death or dementia prior to our baseline and 61 twins whose dementia onset preceded their first record of CVD in the NPR, leaving 2,214 in the final study population. A part of this sample, i.e. like-sexed pairs born 1903-1936 (N=1,053), was also included in the register-based study population (by design).

	Study I	*Study II*	*Study III*	*Study IV*
Design	**Retrospective cohort / cross-sectional**	**Retrospective cohort**	**Case-control**	**Case-control**
Subjects	**Like-sexed twins born 1903-1936 (retrospective cohort) / like- and unlike-sexed twins born before 1935 (cross-sectional)**	**Like- and unlike -sexed twins born before 1936 ("clinical cohort") / like-sexed twins born 1903-1936 ("register cohort")**	**Like- and unlike-sexed twins born before 1936**	**Like- and unlike-sexed twins born before 1936 LOAD CC sample**
N	**22,188 / 7,800**	**2,214 / 18,405**	**nCC: 186 cases and 366 ctrls pCC: 97 cases and 205 ctrls**	**DNA: ~4000 nCC: 179 cases and 364 ctrls pCC: 97 cases and 205 ctrls**
Age at baseline (years), mean (range)	**52.9 (37-71)* / 74.1 (65-103)**	**55.3 (25-87)^/ 60.4* (60-65)**	**nCC: 78.3 (53-92) pCC: 81.5 (67-93)**	**DNA: 77.6 (45-103) nCC: 78.3 (53-92) pCC: 81.5 (67-93)**
Data sources	**Q63, Q67 (Q70), Q73, NPR+CDR, HARMONY**	**Q63, Q67 (Q70), Q73, NPR+CDR, SOG**	**SOG, HARMONY**	**SOG, HARMONY, LOAD CC**
Risk factors	**Asthma, eczema, rhinitis, atopy**	**Non-stroke cardiovascular disease**	**anti-phospholylcholine**	**C-reactive protein, Interleukin-6**
Outcomes	**AD, dementia, survival**	**AD, dementia**	**AD, dementia**	**AD, serum CRP and IL6 levels**
Analysis	**Cox regression, conditional logistic regression (co-twin control analysis)**	**Poisson regression (with GEE), conditional logistic regression (co-twin control analysis)**	**Conditional logistic regression**	**Alternating logistic regression Conditional logistic regression**

Table 1. A summary of study design and analysis for studies I-IV.
nCC: nested case-control, pCC: prevalent case-control, SOG: **S**atsa, **O**cto-Twin, **G**ender, LOAD CC: late onset Alzheimer's disease case-control, NPR: National Patient Register, CDR: Causes of Death Register, GEE: generalized estimating equations, ctrls: controls.
*Baseline for dementia follow-up. ^Baseline for start of exposure data collection.

Information on birth year and sex was defined according to the data in the STR. History of smoking (ever vs. never) and level of education (≤ elementary school vs. > elementary school) was based on twins' self-reports in Q63, Q67 and Q73. BMI (≥25 kg/m^2 vs. <25 kg/m^2) was based on physical measurements and available for 1,662 individuals who participated in the in-person dementia testing sessions. Diabetes and stroke information was derived from the NPR. APOE genotype was available for the 1,623 individuals who donated a blood sample and analyzed as a dichotomous variable (any APOE4 allele vs. no APOE4 allele).

Relative risks were estimated as HRs in a Poisson regression model fitted within a GEE framework to adjust for correlated twin data. CVD was included as a time-dependent variable. Time-at-risk for AD/dementia was calculated as time from baseline to dementia onset, last follow-up occasion, other causes of dementia , or end of study (31st December 2003). The risk for AD/dementia was simultaneously estimated for the first three years since hospitalization and the following years after hospitalization. The cut-off of three years was an arbitrary choice to minimize the time elapsed since the CVD event while maintaining sufficient power for analysis. Co-twin control analyses were based on all twin pairs where at least one individual developed AD/dementia. Only pairs where the partner to the demented proband was alive and non-demented at time the proband developed AD/dementia were included. CVD data on both twins in the pair was collected until the date the proband developed dementia.

Register cohort. The register cohort was compiled and analyzed in a similar manner to the clinical cohort with the exceptions now stated. The inclusion criteria for the register cohort were as follows: 1) born 1903-1936, 2) responded to Q63, Q67 (Q70) or Q73 and answered the questions about cardiovascular disease, 3) alive and living in a Swedish county covered by the NPR by the age of 65.

Q63, Q67 and Q73 included a set of questions regarding chest pain elaborated by Rose[187] to assess AP and that the World Health Organisation in 1963 recommended for use in epidemiological studies. MI was assessed with two questions: ever having had an MI, or ever having had an intense pain across the chest that lasted for half an hour or more. The information in Q63, Q67 and Q73 was used to define baseline exposure status. The questionnaires were also used for self-reported covariate information similar to that in the clinical cohort.

The outcome was hospitalization with AD/dementia. Dementia was ascertained through linkage to the NPR and CDR. A "surrogate" date of dementia hospitalization was calculated for those twins who only had a dementia diagnosis from the CDR (N=153) by deducting three years from the date of death (three years was the average time between first hospitalization with dementia and death with dementia in those 273 individuals with a dementia diagnosis in both the NPR and CDR). Age at start of dementia follow-up ranged from 60 to 65 years depending on when the NPR became complete in the county of residence. The lower age limit (60 years) was defined so that individuals would be at risk of dementia at the start of follow-up. The higher age limit was imposed to minimize the risk of including individuals with dementia at baseline.

Study III: Antibodies against Phosphorylcholine and dementia

In Study III, we evaluated the association between circulating levels of IgM antibodies against phosphorylcholine (anti-PC) and AD/dementia by analyzing selected serum samples from SATSA, OCTO-Twin, GENDER, and HARMONY. The study sample consists of a) a nested case-control study of 182 incident dementia cases matched to 366 controls, and b) a case-control study of 97 prevalent dementia cases matched to 205 controls. Cases were matched to three non-related controls on sex and age at blood draw (± 1 year) and controls could be matched to more than one case.

Nested case-control study. The nested case-control design is an approximation of the full cohort approach.[188] The design is often used in studies of biological precursors of disease since it is a cost- and time-effective method to estimate a relative risk with only minor loss in statistical efficiency compared with an analysis of all subjects included in the full cohort (and without losing the important aspect of prospectively collected exposure information). In this study, the full cohort would equal all individuals in SATSA, OCTO-Twin and GENDER with a cognitive evaluation and blood sampling (before dementia onset for cases). The basis for the selection of cases and controls is creating risk sets. In this study there was no selection of cases since all of the 182 available incident dementia cases (serum collected before dementia onset) with a diagnosis of AD, VaD, mixed AD/VaD, or NUD were included for analysis. Time-matching is an essential feature of this design and controls are selected from among those in the cohort who have not developed the disease by the time of disease onset in the case (referred to as incidence density sampling). Accordingly in our study, individuals who developed dementia were eligible as controls until dementia onset. Controls

are thus selected "longitudinally" during the course of the study, i.e. from the *person-time* of the study base. Thus, the measure of effect (OR) is an approximation of the rate ratio.

Each risk set contained one case and all eligible controls who fulfilled the matching criteria (i.e. controls who fulfilled the matching criteria to multiple cases were included in multiple risk sets). Three controls were then randomly selected from each risk set. Due to the relatively tight matching on age at blood draw (only ±1 year), there were not always three eligible controls for every case, especially in the very old age strata. All eligible controls in these risk sets were then included (without random sampling). In total, the 182 dementia cases were matched to 366 unique controls, of whom 55 (14.5%) later became dementia cases themselves and thus also are included in the case population of 182 individuals.

Prevalent case-control study. The primary intent of the prevalent case-control study was to compare anti-PC levels in those with dementia compared to those without. One hundred and five prevalent dementia cases (serum collected after dementia onset) with an AD, VaD, or mixed AD/VaD diagnosis were randomly selected from SATSA, OCTO-Twin, GENDER and HARMONY. The selected cases (of whom 97 individuals had at least one eligible control and enough serum sample available to allow for reliable analysis) were then matched to three non-related controls on sex and age at blood draw (of whom 205 unique individuals had enough serum for analysis). In order to have a control sample that was not likely to include individuals in a pre-clinical phase of dementia, controls were selected with cumulative incidence sampling, i.e. from those free of dementia at the end of the study. HARMONY participants were not considered eligible as controls due to the selected nature of the HARMONY sample (in principal, only twins who were suspect of dementia, or a twin partner to a person suspect of dementia, were invited to blood sampling).

Exposure definition. Anti-PC was analyzed as quantiles (tertiles, quartiles, quintiles and deciles). Quantile cut-offs were based on the anti-PC distribution in the control groups. The associations between serum levels of anti-PC and incident or prevalent dementia were determined by conditional logistic regression models with calculation of odds ratios (ORs) and 95% confidence intervals (CI). Age and gender were matched for by design of the study. Skewed variables were log-transformed (natural logarithm) for a better normal approximation. Linear trend was assessed through including ordinal variables as continuous variables in a logistic regression model.

Study IV: CRP, IL6 and Alzheimer's disease

In Study IV we aimed to study DNA sequence variation and serum levels of CRP and IL6 in relation to dementia in general and AD in particular.

Serum. The study design for the study on serum levels of hsCRP and IL6 in relation to AD/dementia was identical to the study design for analysis of anti-PC in Study III (described in detail above). Serum was simultaneously aliquoted for analysis of anti-PC, hsCRP and IL6. When there was little sample left, priority was given to anti-PC. Hence, the nested case-control study of hsCRP/IL6 had 179 incident dementia cases (serum collected before onset of dementia) matched to 364 controls. There was however no difference in numbers for the prevalent case-control study.

DNA. In total, DNA was available from 1,567 incident and prevalent dementia cases (of whom 1,265 with AD) and 2,370 controls. Samples were derived primarily from twins in the STR (participants of SATSA, OCTO-Twin, GENDER and HARMONY) but also included an independent non-twin case–control Swedish AD sample, LOAD CC (described in detail above).

Genotype-tagging single-nucleotide polymorphisms (tagSNPs) around *CRP* on chromosome 1 (including 17 kbp upstream of the transcription start site and 6 kbp downstream of the transcription end site) and *IL6* on chromosome 7 (including 10 kbp upstream and 6 kbp downstream) were selected with HaploView[189] 4.1 Tagger[190] using LD and the CEU HapMap population. The tagSNPs included in the study were selected to capture as much genetic variation in the region as possible. Tagging criteria were as follows: Hardy-Weinberg Equilibrium (HWE) p-value cutoff= 0.05, minimum genotyping success rate= 90%, maximum numbers of Mendelian inheritance errors=1, minor allele frequency (MAF)>=0.05, r2>=0.95. Markers that had previously been reported to be associated with cognition-related outcomes were included even if they did not match these criteria. SNPs in perfect LD with SNPs previously reported to be associated with risk of AD were also included as a "back-up" in case of genotyping difficulties. Illumina SNP design scores were calculated by an algorithm developed by the company that predicts success of the assay for the marker. Marker rs1800797 in IL6 did not satisfy the criteria for Illumina probe chemistry and was removed from the list of SNPs to be genotyped. There was no SNP in perfect LD for replacement.

The 22 SNPs included in this study are as follows (presented as dbSNP number and position alias relative to GenBank accession number AF449713); for *CRP*: rs2794520 (7289 G>A), rs1205 (3872 G>A), rs1800947 (2667 G>C), rs1417938 (1919 A>T), rs3116650 (-12976 A>G), rs11265260 (-13934 A>G); for *IL6*: rs11766273 (8844 G>A), rs10242595 (7412 G>A), rs2069861 (4835 G>A), rs1554606 (1888 A>C), rs2069840 (1753 C>G), rs1474347 (1305 A>C), rs2069837 (1208 G>A), rs1800795 (-174 G>C), rs1800796 (-399 G>C), rs2069827 (-1363 C>A), rs12700386 (-3810 C>G), rs2056576 (-5617 G>A), rs10499563 (-6331 A>G), rs1880241 (-7350 A>G), rs7801617 (-8737 G>A), rs1546762 (-9316 G>A).

HWE for individual loci was assessed using the Pearson $\chi 2$ statistic. ALR was used to account for relatedness in the twin sample and generate odds ratios (OR) and 95% confidence intervals (CI) when analyzing alleles and genotypes. Tests of genotypes versus quantitative traits (e.g. age of dementia onset) were conducted using analysis of variance (ANOVA). Haplotypes were estimated after LD block definition[191] in individual blocks using Haploview v4.1 which was also used for statistical analyses. Statistical significance was considered at an overall α=0.05. Adjustments for multiple testing included the Bonferroni correction with a study specific significance threshold at 0.002 for each test (p=0.05 divided by number of tests, i.e. the 22 SNPs) and permutation tests for haplotype data.

5 RESULTS and DISCUSSION

Study I: Atopic disorders and Risk for Dementia

Study I aimed at investigating the association between inflammatory atopic disorders and AD/dementia.

The cumulative prevalence of atopic disorders among the participants of the study ranged from 3.2% for asthma, to 9.0% for eczema and 15.1% for rhinitis. In total, 19.0% reported having or having had asthma, eczema, and/or rhinitis. The cumulative prevalence of atopic disorder was somewhat lower in this material compared to other studies.[90, 192, 193] This can be explained by our relatively old cohort as well as the exposure assessment being based on a single question instead of on a symptom based approach. Data on atopy was collected when the study participants were between 36 and 67 years of age and it does thus represent a possible mix of childhood, adolescent and adult disease. The prevalence and incidence of atopic disorders is highest in childhood, e.g. approximately 85% of individuals affected with dermatitis have their onset before 5 years of age.[90] However, many people grow out of the disease. A diagnosis of disease from a questionnaire is, understandably, often less exact than a physician's diagnosis. The reliability and validity of the current diagnoses have previously been evaluated based on Q63 and Q67.[194] Asthma and eczema had a high agreement with a physician's diagnosis (kappa coefficient 0.84 and 0.87, respectively) whereas rhinitis did not (kappa 0.57).

In the longitudinal setting, dementia diagnoses were ascertained through linkage to the NPR and CDR. Twins were on average 52.9 (range 37.0-71.0) years at the start of follow-up (January 1st, 1974), 78.7 years when first hospitalized with dementia, and 82.4 years when deceased in dementia. Of the 22,188 twins included in the study population, 1,332 (6.0%) were diagnosed with dementia until the end of follow-up (31st December, 2001). Of dementia cases, 887 (66.6%) had a diagnosis of AD. The incidence rate of dementia was 2.66 cases per 1,000 person-years. This is substantially lower than reported by other studies [1, 195, 196] where incidence rates range from approximately 5 to 100 cases per 1,000 person-years depending on age and geographic region. Probable explanations for the discrepancy include our incorporation of relatively young individuals (and thus at a very low risk of

dementia), the incomplete coverage of the NPR until 1987, as well as the low sensitivity (approximately 40%) of the NPR and CDR to register cases of dementia.[197] Including young individuals at a very low risk of dementia will add few cases in proportion to the amount of added follow-up time. This will affect absolute measures of incidence but not measures of relative risks. The incomplete coverage of the NPR between 1974 and 1987 will lead to some dementia cases being missed, i.e. misclassified as non-demented. However, this misclassification would most likely be non-differential in regards to atopic status and thus lead to a bias toward the null.

Atopy increased the risk of dementia by 16% (95%CI 1-33%) in the longitudinal analysis adjusting for age, sex, history of smoking, level of education and non-fatal myocardial infarctions. The relative risk of AD was very similar to that for dementia, but with wider confidence intervals due to the smaller sample size, HR 1.16 (0.98-1.37). There were no elevated risks with asthma or rhinitis alone. Eczema was not a risk factor in the full cohort analysis but a risk factor in co-twin control analysis; a twin diagnosed with dementia was approximately four times more likely to have had eczema than was the non-demented identical twin partner (OR 4.18, 1.35-12.97). The elevated risk in co-twin control indicates that it is the eczema per se that increases the risk of dementia since identical twins are perfectly matched on genes and early environment. However, any differences in results between the full cohort case-control analysis and co-twin control analysis should be interpreted with caution given that they were not statistically significant from another.

In the cross-sectional study, the exposure data was identical to the data used in the longitudinal study whereas dementia was assessed in HARMONY (1998-2001) with clinical work-ups and neuropsychiatric assessment (instead of register linkage). Of the 7,800 individuals above 65 years included in the study, 458 (5.9%) were classified as having a non-secondary dementia (of which 303, or 66.2%, with a diagnosis of AD). In multivariate logistic regression analysis adjusting for age, sex, level of education and history of smoking, there were no significant findings for asthma, eczema, rhinitis, or any atopy with either AD alone or all dementias. Overall, the risk estimates were lower in the cross-sectional study compared to the longitudinal study, with the lowest risk estimate observed for asthma and AD, OR 0.30 (96%CI 0.09-1.04).There were no significant findings in co-twin control analysis.

Overall, there was an indication of a poorer survival for AD cases with asthma. We also found asthma (irrespective of AD) to be significantly associated with shorter survival which is in line with previous reports.[198, 199] Based on the 776 cases with an AD diagnosis in the NPR, the HR of death due to a history of asthma was 1.45 (0.89-2.37). Also indicative of worse survival prognosis in asthmatic AD cases was the finding in the cross-sectional study that only 22.2% of the asthmatics had a date of dementia onset more than 3 years prior to being included in HARMONY, compared to 59.1% of the non-asthmatics (p=0.04). However, none of the estimates in the longitudinal analysis reached statistical significance and great caution is therefore warranted in the interpretation. The suggestion of a survival disadvantage in AD due to asthma might simply be a chance finding explained by the small sample sizes for these analyses. Still, if one were to speculate, possible other causes could include forgetfulness to manage asthma medications and/or co-morbidity with chronic obstructive pulmonary disease (COPD)/misdiagnosis of COPD as asthma.

Taken together, these findings indicate that atopic manifestations could increase the risk of developing AD and/or dementia. Still, the borderline significance of the results, the small elevations in relative risk estimates and the lack of published corroborating studies in other populations makes it difficult to refute the possibility that the results can also be explained by residual confounding or chance.

Study II: CVD and Alzheimer's disease

Study II aimed at assessing the association of non-stroke cardiovascular disease with AD/dementia in two cohorts with clinical- and register-based dementia diagnosis, respectively.

Clinical cohort. Initially, we included all 2,287 twins who had been evaluated for dementia in SATSA, OCTO-TWIN, or GENDER. Twelve individuals were excluded due to death or dementia onset before 1st January, 1974 (i.e. baseline for exposure data collection) and 61 individuals were excluded due to dementia onset prior to a CVD diagnosis. The latter exclusion criterion was introduced due to the etiology of CVD. The main cause of CVD in the general population is atherosclerosis. Also, in this study, the CVD diagnosis extracted from the NPR were those with most correlation with atherosclerosis. Atherosclerosis is a slow process that takes years to develop into clinical disease. Dementia cases who later develop CVD are thus more likely to have a higher burden of atherosclerosis and/or more rupture prone plaques than dementia cases who do not develop CVD.

This was also noted in our material as an elevated risk of dementia in the excluded 61 individuals (with CVD before dementia onset) compared to the included 72 individuals with CVD after dementia onset, HR of 1.43 (0.96-2.14), p=0.079 (age-adjusted Poisson model).

Of the 2,214 followed for CVD through linkage to the NPR (1st January 1974 to 31st December 2003), 409 (18.5%) were hospitalized at least once with a CVD related diagnosis. Of the hospitalized individuals, 249 (60.9%) were at some point hospitalized with AP and 222 (54.2%) with MI. There were only 53 individuals (13.0%) that only had hospitalizations for one of the other diagnosis included.

CVD was associated with an almost doubled risk of dementia during the first three years subsequent to hospitalization; HR 1.83 (1.23-2.72) (adjusted for age). By differential diagnosis for dementia, the relative risk was 3.64 (2.01-6.57) for VaD and 1.48 (0.83-2.64) for AD. As expected, CVD was a strong risk factor for vascular dementia. The risk of developing any dementia, AD or VaD, decreased as the time since the CVD event increased. The hazard ratio of the interaction term was 0.97, p=0.22. Although not significant, this is in accordance with the findings in the register cohort (see below) and with findings from the Rotterdam-study.[110]

APOE4. The APOE4 allele is the most well-established genetic risk factor for AD. APOE4 has also been considered a risk factor for CVD[200] although the relative magnitude of this association has recently been refuted in other studies.[201] We tested the effect of APOE4 on the association between CVD and AD/dementia in several ways. First, we included APOE4 carrier status as a covariate in the age-adjusted Poisson model but without an effect on our estimates. Second, we stratified on APOE4 carrier status. This disclosed that the association between CVD and AD/dementia was restricted to APOE4 carriers: for all dementias, HR 2.37 (1.34-4.22) and 1.45 (0.76-2.75) for APOE4 carriers and non-carriers, respectively; for AD, HR 2.39 (1.15-4.96) and 0.76 (0.24-2.42), for APOE4 carriers and non-carriers, respectively. Risk estimates for VaD were unaltered by APOE4 carrier status. Third, to test whether our findings were related to the effect of APOE4 on timing or severity of CVD we looked at age at first hospitalization with CVD and number of hospitalization occasions with CVD, but will null results. APOE4 carriers and non-carriers were both hospitalized with their first CVD around 74 and a half years and they were also equally admitted to hospital with CVD, on average 1.65 times for non-carriers and 1.52 times for carriers (p=0.53). Although testing the effect of *APOE4 on CVD*

risk was not in the scope of this study, the relative risk of CVD due to APOE4 was also modeled but with null results (HR 0.95, 0.73-1.23, p=0.68). Combined, these results indicate that APOE4 is not a confounder of the association between CVD and AD/dementia, nor that it is in the causal pathway. However, the interaction between APOE4 and CVD on dementia risk indicates that APOE4 carriers have an increased susceptibility to the negative effects of CVD.

Register cohort. We also analyzed the association of CVD and MI with dementia in a longitudinal setting with register-based dementia diagnosis ascertained through linkage to the NPR and CDR. Compared to the clinical cohort, the register cohort was 5.1 years older at baseline (p<0.0001), 3.8 years younger at first CVD (p<0.0001), 1.7 years younger at dementia onset (as defined by hospitalization with dementia) (p<0.0001), 7.8 years younger at death (p<0.0001), had a higher burden of CVD (p<0.0001), a larger proportion of men (p<0.0001) and ever smokers (p<0.0001). The discrepancy in age is explained by the inclusion criteria of the register cohort, that all participants had to be 60 years or older at baseline. The differences between the clinical and register cohorts in age at first CVD, age at dementia onset, age at death, CVD burden, and proportion of men and ever smokers, can be explained in terms of survival and determinants of study participation. In the clinical cohort study participants had to survive to relatively high ages to be considered for inclusion. It is also known that Individuals with a high degree of morbidity are less likely to agree to study participation. The register cohort is therefore likely to be more representative of the Swedish general population.

In the register cohort, the relative risk of dementia due to CVD was 1.98 (1.57-2.49) during the first three years after hospitalization with CVD, 1.43 (1.20-1.72) during the following 10 years, and 1.25 (1.04-1.50) thereafter. The effect of CVD on dementia risk decreased significantly by a factor of 0.97 (p<0.0001) with longer follow-up times since CVD. Given that there is a known lag-phase from clinical dementia onset to first hospitalization with dementia,[197] it is reasonable to assume that a proportion of these individuals had had their dementia onset before the CVD event. It is thus likely that the present risks are affected by a “diagnostic” bias (bringing already demented individuals to the attention of the NPR due to their CVD hospitalization). However, an alternative, or additional, explanation is that the CVD event will trigger a conversion of sub-clinical dementia to clinical dementia. This latter explanation is supported by the fact that we see similar effects in the clinical cohort, a cohort where the dementia diagnosis is not dependent on hospitalizations. To test the

existence of non-differential outcome misclassification in the register cohort (i.e. a higher degree of dementia ascertainment in exposed compared to non-exposed) we also reran our analysis including only dementia diagnosis as the primary cause of hospitalization as cases, thus excluding the 356 cases with only a secondary NPR diagnosis. The relative risk of AD decreased with approximately 20% whereas the relative risk of VaD remained relatively unaltered by the re-analysis.

Familial effects. In co-twin control analysis in the clinical cohort (and the register cohort), the twin with dementia was more likely to have had CVD than was the non-demented identical or fraternal twin partner who had survived to the same age (OR 1.86, 1.11-3.13). A twin with VaD was more than 3 times more likely to have had CVD than his or her non-demented partner whereas a twin with AD was not more likely to have had CVD than the twin partner. The estimates generated in the analysis of the full cohort and the analysis within twin pairs are very similar, indicating that the association is not confounded by unmeasured familial factors in common to both CVD and AD/dementia (e.g. genes or early behavior traits). In other words, it appears to be the CVD (or the CVD-associated disease process) that mediates the increased risk of dementia. This also provides a rational for treating CVD as a possible means to reduce dementia risk.

Stroke. Stroke was not included in our definition of CVD. However, CVD is a risk factor for stroke[202] and, in our material, stroke was approximately two times more prevalent in the CVD group than the non-CVD group, in both the clinical and the register cohorts ($p<0.0001$). To test whether our association between CVD and dementia was affected by stroke, we included stroke as a covariate in multivariate analysis, excluded prevalent stroke cases and censored incident stroke cases. These adjustments had no, or marginal, effects on the relative risk of AD or any dementia.

In summary, the results in Study II indicate that clinical CVD is a risk factor for AD/dementia in APOE4 carriers and that the risk is not mediated or confounded by sharing of genetic or early environmental factors in common to both CVD and dementia.

Study III: Antibodies against Phosphorylcholine and dementia

Study III aimed at investigating the association of circulating levels of antibodies against phosphorylcholine (anti-PC) with AD and dementia. The study included two parts, a nested case-

control study addressing the association with incident dementia and a prevalent case-control study addressing the difference in anti-PC levels in dementia patients compared to controls.

Incident dementia. The effect of having low levels of anti-PC on the risk of developing AD and dementia was investigated with a nested case-control design of 182 incident dementia cases matched to 366 unique controls on sex and age at blood draw (±1 year). The risk of dementia was found not to be affected by anti-PC levels as measured on average 4.3 years before dementia onset. The findings were negative irrespective of reference quantile definition. Neither were there any detectable differences in risk of AD/dementia due to anti-PC levels stratified by sex, APOE4 carrier status, or blood pressure. Multivariate analysis adjusting for a variety of potential confounders gave similar negative findings. Performing co-twin control analysis, i.e. comparing the risk of AD/dementia in a twin individual with low anti-PC levels to the risk of dementia in the identical or fraternal twin partner with median or high anti-PC levels, did not generate any significant findings.

Prevalent dementia. Individuals with AD or dementia had a two-fold higher probability of belonging to the lowest anti-PC quartile than were age- and sex-matched controls, OR 2.04 (1.21-3.44). The probability of being affected with AD/dementia increased linearly with decreasing levels of anti-PC (p=0.02). Multivariate analysis including education level, APOE4 carrier status, smoking, BMI, blood pressure, blood pressure medication, diabetes, diabetes medication, myocardial infarction, stroke, blood lipid levels, NSAID use, ASA use, serum hsCRP levels, and IL6 levels did not significantly alter the results. However, stratifying the analysis on gender and APOE4 carrier status, gave at hand that the association between low anti-PC and AD/dementia was restricted to females, with an OR of 3.39 (1.74-6.59) compared to 0.89 (0.32-2.48) for men (p-value for interaction term 0.039), and APOE4 non-carriers, with an OR of 3.63 (1.90-9.92) compared to 0.85 (0.34-2.13) for carriers (p-value interaction term 0.007).

The results in this study are somewhat ambiguous and can be difficult to interpret. We found no increased relative risk of developing AD or dementia in the nested case-control study, whereas we see a clearly increased odds ratio of belonging to a lower anti-PC quartile if you have AD or dementia. Not only are the results from the incident and prevalent studies at odds with each other, we also noted that the findings in the prevalent case-control study was restricted to females and APOE4 non-carriers. There is no single framework that can easily explain the discrepant findings but

factors related to random variability, survival and control sampling schemes could all explain parts of the findings.

Survival. The results from the prevalent case analyses lack temporality and thus preclude inferences on causality. An association between a risk factor and a disease noted in a cross-sectional setting can nonetheless reflect a true risk relation but can also be due to reversed causality, i.e. the disease affects the risk factor. Another possibility is that the risk factor influences survival in the disease which would introduce bias given that prevalent data are affected not only by disease incidence but also by disease duration. A possible interpretation is thus that individuals with other risk factors (e.g. APOE4) are less well equipped to withstand the additional burden of low anti-PC levels, thus removing this subset from the population at a higher pace. However, if survival in dementia was shortened due to having an adverse (i.e. low) anti-PC level, we would have a selection of dementia cases with higher anti-PC levels than in the source population and would thus only have underestimated the association between anti-PC levels and the probability of having AD.

Control sampling. The reason for the discrepancy between the incident and the prevalent studies could be explained by different factors related to study design and the insidious onset of dementia. Although controls were sampled from the same study population in both the incident and the prevalent studies, the selection criteria were slightly different. Anti-PC in prevalent dementia patients were compared to anti-PC in controls who remained in the study without developing dementia whereas anti-PC levels in incident dementia cases were (as imposed by the nested case-control study design) compared to anti-PC in controls who were allowed to (later) develop dementia themselves. Moreover, the onset of dementia is not as clear cut as for e.g. MI and stroke, and it is therefore reasonable to believe that some of the controls in the incident study were in fact in a pre-clinical stage of dementia at time of blood sampling. Furthermore, our study participants were all elderly individuals with mean age of nearly 82 years. It is possible that, as for many other CVD-related risk factors that have also been linked to AD and dementia, measuring anti-PC levels in mid-life would give a different result.

AD vs VaD. We also noted a (non-significant) difference in anti-PC levels between cases of VaD and AD. Perhaps counter intuitively (since high anti-PC levels have been shown to decrease risk of MI and stroke)[176, 203, 204], we saw *higher* anti-PC levels in VaD cases compared to AD cases in both the

incident and the prevalent material. This could potentially reflect differences between VaD and AD, and the involvement of vascular factors in the two disorders.

Anti-PC was also measured in a random sample of non-demented controls followed longitudinally and who had blood sampling from more than one occasion (on average 3.1 years between samples). Anti-PC levels decreased on average 2.1 U/ml and year (p=0.04). The correlation of anti-PC with other continuous variables was investigated both in this smaller sample and in the large group of controls and anti-PC was found not to correlate with blood lipid levels, blood pressure, BMI, hsCRP, or IL6. The lack of a correlation between anti-PC and blood lipid levels and markers of inflammation indicates that whatever the association between anti-PC and AD/dementia is due to, it involves other mechanisms or pathways than those already captured by measuring blood lipids, and hsCRP or IL6.

Study IV: CRP, IL6 and Alzheimer's disease

Study IV addressed the association between the inflammatory markers CRP and IL6 and the association with dementias. The study was multi-facetted and included the scrutiny of both genetic sequence variation and circulating protein levels.

Genetic association study. The genetic endeavor included six SNPs in the CRP region and sixteen in the IL6 region. Two SNPs in CRP (rs1800947 and rs1417938) and three SNPs in IL6 (rs2069861, rs1546762, and rs12700386) showed significant associations with AD before, but not after, correcting for multiple testing. The multiple testing threshold was defined by the Bonferroni correction threshold of 0.002, i.e. by dividing the overall statistical significance threshold of 0.05 with the number genotyped SNPs. Although the Bonferroni correction method is considered conservative[205] one should also bear in mind that the actual number of tests performed is even larger when considering stratified analysis, haplotype analysis etc.

In CRP, the minor allele C at rs1800947 was more common in AD cases than in controls (9.2% and 7.8%, respectively) (p=0.028). At rs1417938, major allele A was more common in cases than controls (70.8% and 68.7%, respectively) (p=0.072). Analysis of the four haplotypes (see manuscript supplement) also revealed that the haplotype incorporating the two risk alleles was the haplotype with the strongest association with AD although, again, the p-value was not significant after

corrections for multiple testing (p=0.08). At the genotype level, there was a linear trend with increasing risk by increasing risk allele dose for both rs1800947 and rs1417938 although minor allele homozygotes for rs1800947 were only seen in approximately 1% of the population making statistical inference difficult.

Interestingly, variation at rs1800947 and rs1417938 was also related to variation in circulating levels of CRP (in concordance with previous studies).[206-208] Minor allele C at rs1800947 (which was more common in AD cases than controls) was related to lower mean levels of CRP when measured in the non-demented control population, G/G = 2.27, G/C = 1.74, C/C = 0.64 mg/L (p=0.004). At rs1417938, minor allele T (which was less common in AD cases than controls) was associated with higher levels of CRP, A/A = 1.91, A/T = 2.43, T/T = 2.34 (p=0.016). Variation at rs1800947 and rs1417938 explained approximately 1% each of the variation in serum levels. In conclusion, genotypes and alleles that were more common in AD cases as compared to controls were related to lower circulating levels of CRP. Another observation worth mentioning regarding rs1800947 and rs1417938 is that their observed genotype distributions deviated significantly from HWE in the control population before (p=0.02 and 0.03, respectively), but not after, considering multiple testing. Deviations from HWE is often interpreted as an indicator of genotyping error at a specific locus but departures from HWE can also be due to underlying biology. A genotype that is associated with a survival benefit in the general population would for instance be enriched in an older population and thus, deviate from HWE. The AD and dementia cases included in this study were a mix of incident and prevalent cases. Therefore, a possible, although speculative, explanation for the association between less “potent” CRP genotypes and AD is that a pro-inflammatory profile in old age is associated with a survival disadvantage, which will deplete the more potent genotypes in an aging population such as ours.

In IL6, the lowest p-value was observed for rs2069861, where minor allele A was less common in cases than controls, 6.8% and 8.5%, respectively (p=0.01). At the genotype level, G/A was associated with a lower risk of AD compared to the major homozygote G/G, OR 0.73 (0.60-0.90). The minor homozygote (A/A) was only observed in less than 1% of the population, making statistical inference difficult. Analyzing haplotypes at the two LD blocks covering the IL6 gene, revealed no significant findings.

None of the investigated SNPs in CRP or IL6 had significantly different distributions between the AD case population and the control population. However, combining the most significant SNP in CRP (rs1800947) and the most significant SNP in IL6 (rs2069861) into an ordinal additive risk score ranging from zero (no risk allele at either locus), to four (homozygote for risk alleles at both loci) gave at hand a joint additive effect of OR 1.24 (1.09-1.41).

Serum case-control studies. Circulating levels of CRP and IL6 were investigated in relation to dementias in a nested case-control study of incident dementia (serum collected on average 4.3 years before dementia onset) and a case-control study of prevalent dementia (serum collected on average 5.5 years after dementia onset).

Neither levels of CRP or IL6 were related to risk of developing AD, VaD or overall dementia. Multivariate analysis including APOE4, BMI, smoking, blood pressure, educational level, diabetes, CHD or stroke did not give different results. Stratifying on the above mentioned covariates also gave only negative findings.

Levels of both CRP and IL6 were related to the probability of being a prevalent dementia case. Circulating CRP levels were found to be elevated in cases with VaD or mixed AD/VaD (but not AD) as compared to non-demented controls, 4.9 and 2.6 mg/L respectively (p=0.01). Mean IL6 levels on the other hand were elevated in all types of dementia cases compared to the 3.0 ng/L in controls; 4.1 ng/L in AD patients, 5.2 in VaD patients, and 4.3 in all dementias (p=0.002, <0.0001, <0.0001, respectively). There was also a positive linear trend of increasing odds ratios with each increase in tertile (p=0.05). Stratified analyses revealed a significant interaction between IL6 level and educational attainment on the probability of having AD, OR 7.83 (2.32-26.51) and 1.48 (0.77- 2.83), for those with more than and less than or equal to elementary school, respectively (p=0.03 for interaction). Similarly for APOE4 carriers and non-carriers, 3.95 (1.56- 9.99) and 1.43 (0.67-3.09) (p=0.07). The association between IL6 (but not CRP) and AD was also stronger in those who had survived longer times with their dementia diagnosis, the association in those below the median years survived from dementia to blood draw (<4.8 years) was OR 1.55 (0.78-3.08), in those above the median (>=4.8 years), OR 2.99 (1.47-6.08).

In summary, genetic variation and circulating levels of CRP and IL6 do not appear to predict AD or dementia when measured late in life. Nonetheless, dementia patients appear to have an altered IL6 and CRP profile compared to age- and sex-matched controls; IL6 and CRP concentrations were elevated in cases of AD and CRP was elevated in VaD.

6 GENERAL DISCUSSION

The manuscripts included in this thesis have addressed different manifestations of inflammation in relation to AD and dementia. The aims were to investigate peripheral manifestations of inflammation. The findings in this study have both added to the previous knowledge base and raised new questions for the role of inflammation in AD. This thesis also points to the importance of considering the epidemiological design when interpreting the results in dementia research.

Despite intensive research, the mechanisms and relative importance of inflammation in AD development and general cognitive functioning is still up for debate. Perhaps a clue to the relative deadlock and contradictory findings is given by the need for simplifying something that is in fact utterly complex: "…AD inflammation research has most often been compartmentalized, with some groups specializing in cytokines, others in complement, chemokines, growth factors, oxidative stress, microglial activation, astrocyte reactivity, or other areas. In fact, however, inflammatory mechanisms are highly interactive and almost never occur in isolation from each other."[33]

In favor of a direct influence of inflammation on cognitive function are the findings that inflammation-associated risk factors are not only related to cognitive parameters in cognitively impaired individuals. Chronic inflammation and BMI have been reported to affect cognitive function also in non-demented individuals and inflammatory biomarkers (including IL6) have been associated with total brain volume[209] and shown to have an inverse correlation with hippocampal grey matter volume in middle age.[210]

The results of this thesis add to the picture of the complexity in the etiology of clinical AD as well as the methodological difficulties of studying late-life disorders. An important finding in AD research and a clue to the long pro-dromal stage of AD, comes from the finding that the effect of exposures on AD risk might be heavily influenced by the timing of the exposure. High BP and obesity appears to be risk factors for AD in mid-life[211-213] whereas they appear to be "protective" factors when measured in late-life.[214] This pattern could explain why we see no predictive value in hsCRP, IL6 or anti-PC in regards to dementia when measured in close proximity to (on average 4.3 years before) dementia onset and why results from previous studies are so discrepant; even reviews of systemic inflammatory markers and risk of cognitive decline or dementia reach different conclusions, stating

that "subjects with high levels of systemic inflammatory markers may be at risk of cognitive decline.",[132] or that findings are “contradictory”,[78] or that “high concentrations are predictive of cognitive decline and dementia”.[215] Inflammatory markers have also been investigated as potential biomarkers of AD (i.e. diagnostic markers of disease) but, again, results are inconsistent.[216, 217] Age (and cognitive status) at measurement of inflammatory load could also explain why, despite the fact that a *high* inflammatory burden has been linked to detrimental effects on cognition, *low* CRP levels have been related to a more rapid decline in AD[218]. Also, patients with mild cognitive impairment (MCI) and with low-risk biomarker profiles for AD actually had higher levels of CRP and IL6 than AD patients.[219] Another interesting finding along the same lines is that the metabolic syndrome (associated with an increased risk of dementia when measured in mid-life) is related to a *de*celerated cognitive decline in very old.[220]

Another aspect that highlights the complexity of inflammation in clinical AD development is the multitude of reported interactions. Several studies have found significant interactions between measures of inflammation and sex, age, cardiovascular disease and APOE4,[221, 222] For example, inflammation has been shown to be a risk factor for dementia but only in those with atherosclerosis,[223] the metabolic syndrome has been associated with cognitive impairment but only in those with high levels of inflammation,[224] and atherosclerosis has been shown to have a more detrimental effect on risk of dementia in APOE4 carriers than non-carriers.[225] In this thesis, we saw different associations between inflammatory factors and AD depending on presence or absence of APOE4. Anti-PC was associated with AD in APOE4 *non-carriers* whereas IL6 was associated with dementia in APOE4 *carriers*. Effect measures from studies of prevalent dementia, will be affected both by etiological and prognostic factors. However, if these discrepant findings were only associated with survival in AD, one would expect the same results for anti-PC and IL6, i.e. that APOE4 carriers are less well equipped to withstand the burden of additional risk factors (i.e. low anti-PC and high IL6, respectively) and thus have a worse prognosis. However, since this is not the case, an alternative interpretation is that anti-PC and IL6 have different etiological relationships with AD or different effects on AD prognosis. Speculatively, a clue to the difference can be provided from animal studies showing that inflammatory signaling pathways can regulate APOE gene expression.[226]

A large proportion of dementia cases have mixed disease with both AD pathology and vascular lesions[115] but the association between vascular factors and neurodegeneration is unclear.[118]. Post-

mortem studies of AD neuropathology have found either no association with measures of cardiovascular disease severity[227, 228] or the opposite, an association between coronary artery disease and AD.[229-231] Reviews of epidemiological studies [108-110, 212, 213, 232-235] conclude that there appears to be an association between CVD and risk factors for CVD with AD but the nature of the association remains unknown[114, 236-238] and that results are inconsistent.[239] Several mechanisms have been proposed. There might be an interaction between cytokine biology and lipid metabolism in neuronal repair and integraty.[240] The acute-phase reactant CRP for instance, appears to possess both pro-atherogenic as well as neurological[241] effects.[215, 241] Endothelial and blood-brain barrier function is also known to be impaired in AD,[242] perhaps induced by vascular factors co-existing with AD. An alternative explanation for the association between CVD and AD includes clinical threshold-lowering effects on dementia,[243] perhaps as a consequence of sub-clinical silent infarcts or stroke.[244 245] In line with this, we observed a high risk of dementia in immediate conjunction to the CVD event, a risk that then declined. We also found evidence of a potential diagnostic bias, prohibiting a (perhaps correct) diagnosis of AD in individuals with CVD. It is thus possible that the contribution of CVD to clinical AD is underestimated.

It is important to bear in mind that this thesis deals with clinical AD. Although clinical diagnoses are known to be highly reflective of AD pathology, clinical AD diagnosis should by no means be seen as proxies of plaques and tangle burden. It might be that clinical AD is a summing up of different factors where AD pathology is just one piece of the puzzle. All factors that will increase the occurrence of the disease or lower the age of onset are of interest in modulating disease risk, regardless of whether these factors increase AD pathology or decrease the clinical threshold.

7 CONCLUSIONS

- Atopic disorders in childhood, adolescence or adulthood may confer an increased risk of AD and dementia.
- Cardiovascular diseases other than stroke doubled the risk of AD and dementia in genetically susceptible individuals carrying the APOE4 allele.
- APOE4 is not a risk factor for non-stroke cardiovascular disease.
- The association between non-stroke CVD and dementia is not mediated by genetic or early environmental factors in common to both dementia and CVD.
- Serum levels of IgM antibodies against phosphorylcholine are decreased in Alzheimer's disease patients compared to healthy controls.
- Serum levels of interleukin-6 are increased in all Alzheimer's disease patients compared to healthy controls.
- Sequence variation in the CRP and IL6 genes are not related to risk of Alzheimer's disease or dementia.

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