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Vascular Risk Factors in Alzheimer's Disease and Mild Cognitive Impairment

A Longitudinal, Population-Based Study

Doctoral dissertation

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ABSTRACT

Dementia is a common and disabling disorder in the elderly. Because of the worldwide aging phenomenon of the population, existing in both developed and developing countries, dementia has a growing public health relevance. The major cause of dementia is Alzheimer's disease (AD). AD is considered to be a multifactorial disease resulting from an interaction between genetic susceptibility and environmental factors. Thus, its prevention is likely to be at least partly possible. To determine interventions that would prevent or delay the onset of AD, we must first identify modifiable risk factors for the disease. There is some evidence that risk factors for vascular diseases may also be risk factors for AD.

The present study clarifies the epidemiology of AD in the general population and in different genders with a focus on modifiable vascular risk factors. The relations between midlife elevated blood pressure (BP) and serum cholesterol levels to the subsequent development of late-life AD and mild cognitive impairment (MCI) are examined. Furthermore, the relative importance and putative interactions between the apolipoprotein E (ApoE) ε4 allele, the most important genetic risk factor for AD, and vascular risk factors in the development of AD are evaluated. Putative gender differences in the risk factor profiles for AD are also elucidated, as well as the relation of estrogen replacement therapy (ERT) to AD and MCI.

Participants of this study were derived from random, population samples studied in one of the surveys carried out in 1972, 1977, 1982, or 1987. After an average follow-up of 21 years, a total of 1449 (73 %) individuals aged 65–79 years participated in the re-examination in 1998. Elevated systolic BP (≥ 160 mmHg) and high serum cholesterol levels (≥ 6.5 mmol/l), and, in particular, the combination of these risks at midlife significantly increased the risk of late-life AD. Elevated serum cholesterol level was also a significant risk factor for MCI, which is considered to be a high-risk state for AD, and the effect of high systolic BP approached significance. The ApoE ε4 allele, elevated midlife cholesterol, and high midlife systolic BP constituted independent risk factors for AD, and the risk related to treatable risk factors – elevated cholesterol and BP – appeared to be greater than the risk related to the ApoE ε4 allele. In addition, the effect of the ApoE ε4 allele as a risk factor for AD was stronger in a subgroup that had not received antihypertensive drug treatment at midlife. Vascular risk factors were observed to be important in the development of AD in both genders, but there were some gender-related differences in the risk factor profiles for AD. Finally, women who had used ERT had a significantly decreased risk of AD and MCI compared to those who had never used ERT.

These results point out that midlife elevated systolic BP and serum cholesterol levels have an important role in the development of AD, and emphasise the need for clinical interventions to control these risk factors more effectively. Early interventions aimed at reducing these vascular risk factors are likely to have an impact on the future incidence and prevalence of AD. The finding that the risk related to modifiable vascular factors appeared to be greater than the risk associated with the ApoE ε4 allele, and that the effect of the ApoE ε4 allele was not uniform but may be modified for instance by antihypertensive drugs, as well as the finding concerning the protective effect of ERT against MCI and AD, provide an optimistic outlook concerning future prevention strategies for AD.

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Medical Subject Headings: Alzheimer disease; Alzheimer disease/prevention&control; dementia; cognition disorders; risk factors; hypertension; hypercholesterolemia/blood; apolipoprotein E/genetics; aged; middle age; human; longitudinal studies; follow-up studies; random allocation; Finland

"Whoever labors on a difficult task can easily become despaired and discouraged if they only look ahead and see new obstacles that stand in they way of progress. One has to occasionally glance backward to view the distance already traveled... This in turn will benefit our future work. Because not excessive doubt and immobilizing despair help science move forward but instead a healthy optimism and great confidence in the search for new ways to find knowledge since they will certainly be found."

- Alois Alzheimer, 1913 -

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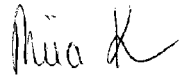

Miia Kivipelto

ABBREVIATIONS

AD	Alzheimer's disease
Aβ	β-amyloid
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
BBB	Blood brain barrier
BMI	Body mass index
BP	Blood pressure
CDR	Clinical Dementia Rating
CI	Confidence interval
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computerised tomography
DLB	Dementia with Lewy Bodies
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th edition
ERT	Estrogen replacement therapy
FTD	Frontotemporal dementia
HDL	High-density lipoprotein
HIS	Hachinski Ischemic Score
LDL	Low-density lipoprotein
MCADRC	Mayo Clinic Alzheimer's Disease Research Center
MCI	Mild cognitive impairment
MI	Myocardial infarction
MMSE	Mini-Mental State Examination
MRI	Magnetic resonance imaging
NFT	Neurofibrillary tangle
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
NINDS-AIREN	National Institute of Neurological Disorders and Stroke - Association Internationale pour la Recherche et l'Enseignement en Neurosciences
NP	Neuritic plaque
OR	Odds ratio
RR	Risk ratio
SD	Standard deviation
VaD	Vascular dementia
VLDL	Very low-density lipoprotein
WHO	World Health Organization
WML	White matter lesion

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications that are referred to in the text by the Roman numerals **I-IV**.

I **Kivipelto M**, Helkala E-L, Laakso MP, Hänninen T, Hallikainen M, Alhainen K, Soininen H, Tuomilehto J, Nissinen A. Midlife vascular risk factors and Alzheimer's disease in later life: longitudinal, population-based study. *British Medical Journal* 2001;322:1447-1551

II **Kivipelto M**, Helkala E-L, Hänninen T, Laakso MP, Hallikainen M, Alhainen K, Soininen H, Tuomilehto J, Nissinen A. Midlife vascular risk factors and late-life mild cognitive impairment. A population-based study. *Neurology* 2001;56:1683-1689

III **Kivipelto M**, Helkala E-L, Laakso MP, Hänninen T, Hallikainen M, Alhainen K, Iivonen S, Mannermaa A, Tuomilehto J, Nissinen A, Soininen H. Apolipoprotein E ε4 allele, elevated midlife total cholesterol level, and high midlife systolic blood pressure are independent risk factors for late-life Alzheimer disease. *Annals of Internal Medicine* 2002;137:149-155

IV **Kivipelto M**, Helkala E-L, Nissinen A, Soininen H, Tuomilehto J. Vascular risk factors, ApoE ε4 allele and gender and the risk of Alzheimer's disease: Perspectives on prevention. *Drug Development Research* 2002 (In press)

V **Kivipelto M,** Helkala E-L, Laakso MP, Hänninen T, Hallikainen M, Alhainen K, Iivonen S, Mannermaa A, Tuomilehto J, Nissinen A, Soininen H. Estrogen replacement therapy and the risk of mild cognitive impairment and Alzheimer's disease. A population-based study. (Submitted)

CONTENTS

1 INTRODUCTION

As the longevity of the population increases, Alzheimer's disease (AD) is becoming an increasingly enormous public health problem. It has been estimated that the number of AD patients will quadruple by 2050 unless a means for prevention or cure is found. From the same projections, it has been proposed that interventions that could postpone disease onset by five years would decrease the projected prevalence of AD by 50 % (Brookmeyer et al., 1998). So far, few strategies are available for the prevention of AD in elderly persons.

AD is considered to be a disease of multifactorial origin, likely to be resulting from an interaction between genetic susceptibility and environmental risk factors. At the moment, we do not know exactly what the initiators and promoters, the real causes underlying AD are. While the genetic constitution of an individual is not modifiable, various environmental risk factors for AD have been proposed, although there is no unequivocal agreement about their importance.

It has been suggested that risk factors for vascular diseases also have a role in the development of AD. Hypertension and hypercholesterolemia especially have recently attracted considerable attention as potential modifiable risk factors for AD. However, most of the epidemiological studies on this issue so far have been cross-sectional or with a relatively short follow-up time, and they have yielded quite controversial results. Long-term prospective studies examining the relation of hypertension and hypercholesterolemia earlier in life to the development of late-life AD in representative populations are few. Because neurodegenerative processes of AD may already begin at midlife (Braak et al., 1999), identification of early risk factors may shed some light on the pathophysiology of AD and also provide avenues for prevention and treatment.

Vascular factors may also be important in the development of cognitive impairment. However, results obtained from previous studies also remain somewhat controversial. Furthermore, in these studies, cognitive impairment has been defined by a variety of neuropsychological tests instead of being based on clinical or diagnostic concepts. Recent research has identified a transitional state between the cognitive changes of normal aging and very early AD, known as mild cognitive impairment (MCI) (Petersen et al., 1999). MCI is

considered to represent a clinically identifiable entity and to be a high-risk condition for AD (Petersen et al., 2001 a). However, past attempts to describe MCI have been primarily clinic-based and the epidemiology of MCI including its prevalence and its risk factors (e.g. vascular) remains largely unknown.

To date, the only genetic risk factor for AD of established general significance is the ε4 allele of apolipoprotein E (ApoE). ApoE is known to be associated with cholesterol metabolism, but the very mechanisms relating the ApoE ε4 allele to AD are not completely understood (Mahley et al., 2000). Similarly, the relative importance and putative interactions between ApoE and vascular risk factors for the development of AD in the general population remain to be established.

The burden of AD falls more heavily on women than men. The prevalence of AD is reported to be higher in women, even after controlling for their higher longevity. However, the role of gender in the development of AD is still controversial, as are the putative gender-related properties that may predispose or protect against the development of AD (Ruitenberg et al., 2001 b). An important gender-related difference that has been associated with the risk of AD is the dramatic decline of women's serum estrogen levels after menopause (Monk et al., 2000). Previous epidemiological studies testing a putative risk reduction of cognitive impairment and AD due to estrogen replacement therapy (ERT) have, however, yielded inconsistent results (LeBlanc et al., 2001).

Interventions that would delay the onset of AD even modestly would have a major impact on public health. Thus, it is important to try to identify modifiable risk factors for AD. The present set of studies clarifies the epidemiology of AD in the general population with a special focus on modifiable vascular risk factors. A combination of a longitudinal, population-based study cohort with a mean follow-up time of 21 years and modern techniques and methods for analyses and diagnoses provided an unique opportunity to conduct the present study. The relationship between midlife elevated blood pressure (BP) and serum cholesterol levels to the subsequent development of late-life AD were examined in a prospective, population-based sample. Similarly, the impact of these risk factors on the development of MCI was investigated, as well as the prevalence of MCI in this elderly, Finnish, population-based sample. The relative importance and the putative interactions

between these vascular factors and the ApoE ε4 allele as risk factors for AD were analysed. In addition, putative gender differences in the risk of AD related to ApoE ε4, midlife elevated cholesterol and BP, and vascular disease were evaluated. Furthermore, the relation of ERT with AD and MCI was examined in women.

2 REVIEW OF LITERATURE

2.1 Dementia and Alzheimer's disease

2.1.1 Definition of dementia

Dementia, derived from the Latin "*de mens*", ie., "*without mind*" is a clinical syndrome of mental impairment produced by brain dysfunction or brain damage. The word dementia is a generic term that has been given different meanings in different contexts; dementia may denote a clinical syndrome, and also imply that the etiology of this syndrome is organic brain disease. Moreover, dementia may be used in a wider sense, describing the underlying brain disease from its early, subtle manifestations to the advanced state of severe deterioration (Gustafson, 2000).

There are several widely used sets of criteria for the diagnosis of dementia. These criteria have conceptual differences and consequently are not interchangeable (Erkinjuntti et al., 1997). One widely used definition of dementia has been given in the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, in the version III (DSM-III) (American Psychiatric Association, 1980), in the revised third version (DSM-III-R) (American Psychiatric Association, 1987), and in the version IV (DSM-IV) (American Psychiatric Association, 1994). The essential feature of dementia according to the DSM-IV criteria is the development of multiple cognitive deficits that include memory impairment and at least one of the following cognitive disturbances: aphasia, apraxia, agnosia, or disturbance in executive functioning. Thus, memory impairment is required to make a diagnosis of dementia. To fulfil the criteria for dementia, the cognitive decline must be sufficiently severe to cause impairment in occupational or social functioning, and it must represent a decline from a previous level of functioning. A diagnosis of dementia should not be made if the cognitive deficits occur exclusively during the course of a delirium. However, dementia and delirium may both be diagnosed if the dementia is present at times when the delirium is not present. The DSM-IV definition of dementia is based on the pattern of cognitive deficits and carries no connotations concerning prognosis. Dementia may be progressive, static, or remitting (American Psychiatric Association, 1994).

2.1.2 Historical overview

Cerebral arteriosclerosis was considered to be a major cause of organic dementia for a long period of time. In the late nineteenth century, new histopathological techniques made it possible to speculate on independent circulatory and degenerative changes behind the development of organic dementia (Gustafson, 2000). It was Alois Alzheimer and Otto Binswanger who revealed the clinical and pathological heterogeneity of dementia caused by vascular disorder (Román, 2002). In 1906 and 1907, Alzheimer described a patient, a 51-year-old woman, who had shown progressive cognitive impairment, focal symptoms, hallucinations, delusions, and psychosocial incompetence. At autopsy, there were plaques and neurofibrillary tangles (NFT) and arteriosclerotic changes (Maurer et al., 1997). This was concluded to be a new form of dementia, and later Emil Kraepelin introduced this disease as Alzheimer's disease (AD) in the category of presenile dementias in the 1910 edition of his textbook (Román, 2002). This was followed by a long period of silence, where AD was considered to be an academic rarity. In the end of the 1960's Blessed, Tomlinson, and Roth showed, in their systemic neuropathological obduction series, that histopathological brain changes described by Alzheimer were very common in elderly demented patients (Blessed et al., 1968). AD was observed to be the most common cause of dementia among the elderly, accounting for 60-70 % of the cases, and in the past 30 years AD has dominated the field of dementia research.

Dementia research has been strongly influenced by the distinction between vascular and degenerative diseases (Gustafson, 2000). This demarcation has probably limited the possibilities of finding risk factors exclusive to each entity. The research advances have since revealed the complexity and etiological, clinical, and pathological heterogeneity of both AD and vascular dementia (VaD). Interestingly, vascular factors have regained an increasing amount of interest recently in dementia research because they have been found to be common phenomena in almost all types of organic dementia. It is now also recognised that VaD extends beyond the traditional concept of multi-infarct dementia (Erkinjuntti, 2002). Recently, there has been discussion about the need for revising the current criteria for dementia and the conventional concepts of primarily degenerative versus vascular forms of dementia (Neuropathology Group of the Medical Research Council Cognitive Function and Aging Study, MRC CFAS, 2001).

2.1.3 Alzheimer's disease – from clinics to neuropathology

Dementia has become a focus of both great public interest and research during the past decades as the world population is aging. Today dementia, and particularly AD, is recognised as a major cause of disability (Green et al., 1993; Aguero-Torres et al., 1998), institutionalisation (Jagger et al., 2000; Aguero-Torres et al., 2001), and mortality (Aguero-Torres et al., 1999) in the elderly, and a cause of immense distress among family members and caregivers.

AD is a progressive neurodegenerative disease with certain characteristic clinical and pathological features. However, AD is etiologically heterogeneous and clinical variations are common, including differences in age of onset, rate of progression, patterns of neuropsychological deficits, and occurrence of neuropsychiatric symptoms.

The typical clinical profile of AD is characterised by insidious onset and progressive decline of memory and other cognitive functions. The disease progresses from mild to moderate and severe status with increasing severity of symptoms, and eventually leads to death. The expected survival time after the disease onset is estimated to be between five to twelve years, and causes of death in AD include infectious diseases such as pneumonia and sepsis, and other common causes of mortality in the elderly such as cardiovascular disease and stroke (Friedland, 1993; Beard et al., 1996). However, a recent study reported much shorter median survival after the onset of dementia (adjusted median survival 3.3 years, 95 % confidence interval (CI) 2.7-4.0 years), similar to that of other serious diseases in older persons (e.g. heart failure), which thus highlighted the deleterious effects of disease (Wolfson et al., 2001).

Although cerebral atrophy is a typical manifestation of AD, it does not distinguish normal aging from AD accurately enough for differential diagnoses; this applies to neuroimaging as well as gross inspection at *post mortem* (Munoz and Feldman, 2000). However, microscopic examination reveals the hallmark features of the disease – a cerebral cortex peppered with amyloid plaques and neurofibrillary tangles (NFT). By definition, the one neuropathological abnormality required for the diagnosis of definite AD is an adequate number of amyloid plaques (Khachaturian, 1985), which forms the basis of current neuropathological diagnostic

criteria for AD, e.g. Neuropathology Task Force of the Consortium to Establish a Registry for Alzheimer's disease (CERAD) (Mirra et al., 1991). Thus, according to these criteria, amyloid has a key role in AD, although the comparative relevance of amyloid plaques on the etiology and pathogenesis of AD is controversial (Poirier et al., 1999).

The β-amyloid (Aβ) peptides that are deposited as extracellular amyloid plaques in the AD brain are formed by the proteolytical processing of the amyloid precursor protein (APP), encoded by chromosome 21. The proteases involved in the proteolytic processing of APP are the α-, β-, and γ-secretases. The formation of Aβ involves the cleavage of APP by β- and γ-secretases, whereas α-secretase cleavage releases a large soluble APP ectodomain (APPα) (Nunan and Small, 2000). The two major types of amyloid plaques in the AD brain are neuritic plaques (NP) and diffuse plaques. NPs contain dense bundles of amyloid fibrils and are surrounded by dystrophic neurites, astrocytes, and microglia. Diffuse plaques contain unstructured amyloid and are not surrounded by dystrophic neurites.

The number of plaques has been reported to be significantly increased in the initial stages of the disease (Haroutunian et al., 1998; Näslund et al., 2000; Morris JC et al., 2001), which supports the hypothesis that Aβ deposition may be an initial pathogenic event in the development of AD. However, whether the Aβ plaques are associated with the severity of dementia has been a matter of controversy. Some studies have found no correlation (Terry et al., 1991; Arriagada et al., 1992) but some recent studies have supported a correlation between Aβ plaques and cognitive impairment (Cummings et al., 1995; Kanne et al., 1998; Näslund et al., 2000). Evidence for a key role of amyloid in the pathogenesis of AD comes from families with APP mutations. Yet, the association of a mutation in APP with AD in these few families does not imply that APP is the initiating factor in the sporadic forms of AD (Poirier et al., 1999). Experimental studies appear to support a role of amyloid in the pathogenesis of AD. Amyloidogenic fragments of APP left in tissue culture medium appear to be toxic to neurons in culture (Yankner et al., 1989). Thus, the deposits of dense amyloid in the AD brain has been suggested to damage the surrounding nerve endings. However, evidence for a pathogenic role of amyloid in AD provided by neuropathological studies is equivocal. A recent study reported an extensive overlap of Alzheimer-type pathology among demented and non-demented older people (Neuropathology Group of the Medical Research Council Cognitive Function and Aging Study, MRC CFAS, 2001), thus suggesting that

additional factors determine whether moderate burdens of Alzheimer-type pathology are associated with cognitive failure, and challenging conventional diagnostic criteria for dementia.

The second neuropathological hallmark of AD is the NFTs found inside neurons. NFTs are composed of paired helical filaments, and to a lesser extent, straight filaments. The main component of paired helical filaments is the abnormally phosphorylated microtubule-associated tau protein (Lee et al., 1991). Braak and Braak (1995) proposed a model for the progression of AD based on the presence of NFTs. During the preclinical phase of the disease, NFTs appear in the entorhinal region spreading then to the hippocampus at the mild phase of the disease and finally to the neocortex during the later stages of AD (Braak and Braak 1995). A correlation between the number of NFTs and cognitive decline in AD has been frequently reported (Wilcock and Esiri, 1982; McKee et al., 1991; Arriagada et al., 1992; Cummings et al., 1996).

Other changes typically found in AD include neuronal and synaptic loss, sequela and markers of oxidative stress and inflammatory processes, membrane alterations, changes in signal transduction (Joseph et al., 2001), microvascular pathology, and vascular lesions (Kalaria and Ballard, 1999; Farkas and Luiten, 2001). The relative importance of each of these pathological changes remains still obscure. In terms of prevention, perhaps the most important question - what are the diverse triggers of these pathological cascades- remains also unknown.

2.1.4 Diagnosis of Alzheimer's disease

There is no agreement on a biological marker that is the most useful in the diagnosis of AD aside from findings on histological examination of the brain. Therefore, the diagnosis of AD is essentially clinical. There are several guidelines for the clinical diagnosis of AD, including the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984), the DSM-IV (American Psychiatric Association, 1994), and the International Classification of Diseases, 10^{th} revision (ICD-10) criteria (World Health Organization, 1993).

The NINCDS-ADRDA criteria have been widely used in research because they are well validated, provide sufficiently high diagnostic accuracy and, being the most widely used, allow comparison between studies (Blacker et al., 1994; Gearing et al., 1995). The NINCDS-ADRDA criteria divide AD into three categories; possible, probable, and definite AD. Differences between definite, probable, and possible reflect the available information (clinical and pathological vs. clinical only) and how closely the patient's syndrome resembles classic AD. The NINCDS-ADRDA criteria for definite AD require that the patient has met clinical criteria for probable AD while living and has histopathological evidence of AD obtained by biopsy or autopsy. The patient has probable AD according to the NINCDS-ADRDA criteria when the presence of dementia has been established by a questionnaire and confirmed by neuropsychological testing, characterised by gradual onset and progression, and when other disorders that could cause dementia are absent. The onset should be between the ages of 40 to 90 years, and no disturbances of consciousness should be present. Features that support the diagnosis of AD but are not required for diagnosis include: progressive deterioration of specific functions such as language (aphasia), motor skills (apraxia), and perception (agnosia); impaired activities of daily living, and altered patterns of behaviour; family history of similar disorders, particularly if confirmed neuropathologically, normal routine cerebrospinal fluid (CSF) samples; normal or non-specific changes on electroencephalography (EEG); and evidence of cerebral atrophy in brain imaging with progression documented by serial observation. Possible AD is diagnosed when the patient has a second brain disorder or systemic illness that is sufficient to produce dementia but is not considered to be the cause of dementia, or when the patient has variations in the presentation of dementia compared with typical AD. The accuracy of the clinical diagnosis of AD using NINCDS-ADRDA criteria has been reported to exceed 80 % (Blacker et al., 1994; Galasko et al., 1994; Gearing et al., 1995; Kosunen et al., 1996; Lopez et al., 1999).

Traditionally it has been stated that AD is a diagnosis of exclusion; currently however, when the typical features of AD are better known, this statement is only partially true (Richards and Hendrie, 1999). While it is essential to evaluate other possible causes of dementia, a positive diagnosis of AD can be made based on characteristic history and clinical examinations. Neuropsychological evaluations and brain imaging give valuable additional information about the disease. Volumetric magnetic resonance imaging (MRI) of

hippocampus and entorhinal cortex may help in the diagnosis of AD. The differentiation of AD from other dementias using hippocampal volumetry, however, has not always achieved a high diagnostic accuracy (Laakso, 2002).

2.2 Mild cognitive impairment – a warning sign of dementia

2.2.1 Concept of cognitive impairment

Normal aging is associated with deterioration of various aspects of cognitive performance. However, mild cognitive problems can also be a sign of incipient AD. The boundary between normal aging and mild AD has become an area of increasing interest for both theoretical and practical reasons (Petersen et al., 2001 a). The identification of individuals at high risk of dementia is important with a view to early therapeutic interventions (Shervin, 2000).

A number of clinical concepts have been proposed to be used in the characterisation of cognitive deficits that do not fulfil the criteria of dementia. Earlier concepts such as benign senescent forgetfulness (Kral et al., 1962), age-associated memory impairment (AAMI) (Crook et al., 1986), aging-associated cognitive decline (AACD) (Levy et al., 1994), and age-related cognitive decline (American Psychiatric Association, 1994) are generally meant to reflect the extremes of normal aging rather than to describe a precursor of pathological aging (Petersen et al., 2001 a). Some studies of these concepts have reported dementia conversion rates that are not different from normal subjects (Hänninen et al., 1995), but some studies have suggested an increased conversion rate (Richards et al., 1999; Ritchie et al., 2001), calling into question the "normality" of these states. One problem with these studies is that the criteria have been inconsistently used, making comparison between studies difficult. Moreover, these concepts are based solely on psychometric criteria, and thus, certain test scores are sufficient for the diagnosis without consideration of their relevance in the individual's life. All considered, the relevance of these concepts in research and clinical practice remains questionable.

Other concepts have been proposed, which link cognitive impairment to pathological states (Ritchie and Touchon, 2000). These include mild cognitive disorder (World Health

Organization, 1993), mild neurocognitive decline (American Psychiatric Association, 1994), cognitive impairment no dementia (CIND) (Graham et al., 1997), and mild cognitive impairment (MCI) (Petersen et al., 1999). In particular, the concept of MCI has recently been a topic of interest among research into aging-related cognitive disorders. MCI is considered to represent a transitional state between normal aging and very early AD, and to be a high-risk condition for the development of dementia, particularly AD. An annual conversion rate of 10 to 15 % to AD in individuals with MCI has been proposed, in contrast to a conversion rate of 1 to 2 % in the normal elderly population of the same age (Petersen et al., 1999). However, the concept of MCI has also been subject to debate, and a recent study proposed that MCI was a poor predictor of dementia in a population-based sample (Ritchie et al., 2001). This study used very stringent criteria for MCI, excluding individuals with impairment in any other area of cognitive function than memory.

2.2.2 Diagnosis of mild cognitive impairment

The criteria of MCI used in many previous studies, especially in the current clinical trials on MCI, have been based on the adaptation of the criteria suggested by the Mayo Clinic Alzheimer's Disease Research Center (MCADRC) (Smith et al., 1996; Petersen et al., 1995; 1999). One of the earliest criteria included the following: 1) memory complaint by patient, family, or physician; 2) normal activities of daily living; 3) normal global cognitive function; 4) objective impairment of memory or in one other area of cognitive function as evidenced by scores >1.5 standard deviation (SD) below the age-appropriate mean; 5) Clinical Dementia Rating (CDR) score of 0.5; and 6) absence of dementia (Smith et al., 1996). Later, the authors made some modifications to these criteria (Petersen et al., 1999), and the current state of MCI was recently summarised by the subcommittee of the American Academy of Neurology (Petersen et al., 2001 a) and a group of experts in aging and MCI (Petersen et al., 2001 b). MCI criteria presented in these contexts differ with some respects from the criteria presented above, including 1) memory complaint preferably corroborated by an informant; 2) objective memory impairment; 3) preserved general cognitive function; 4) intact activities of daily living; 5) not demented. In this latest version of the MCI criteria, the CDR score 0.5 was omitted. This type of amnestic MCI has been the main focus of interest in the MCADRC, and is seen as a precursor for AD (Petersen et al., 2001 b). However, different

definitions of MCI, heterogeneity of the group, and other possible subsets of MCI besides the amnesic group still appear to be subject of discussion (Petersen et al., 2001 b).

It is important to note that the past attempts to describe MCI have been primarily clinic-based, and there have been few studies from representative general populations. Thus, epidemiology including incidence, prevalence, and risk factors of MCI remain largely unknown.

2.3 Epidemiology of Alzheimer's disease

Early epidemiological studies concerning dementia and specific types of dementia were carried out during the 1960s in the Scandinavian countries and in the United Kingdom, but epidemiological methods first became widely used during the 1980's and 1990's in dementia research. During the last decade, epidemiological studies have contributed greatly to the understanding of the etiology of dementia and AD (Fratiglioni and Rocca, 2001). One of the ultimate objectives in epidemiological studies is to develop strategies for the prevention of disease. Despite the progress in the field of the epidemiology of AD, we still do not know what the real causes, initiators and promoters of the disease are, and hence we lack proper preventive strategies for AD.

2.3.1 Prevalence and incidence

The occurrence of a disease can be expressed as prevalence (the proportion of subjects with a given disease in a defined population at a given point of time), or as incidence (the rate of new cases developing the disease in a defined population during a defined time interval). The prevalence is determined both by incidence and disease duration. Most of our knowledge about the occurrence of dementia is based on prevalence rather than incidence data (Fratiglioni and Rocca, 2001).

The prevalence estimates of dementia derived from five meta-analyses (Jorm et al., 1987; Hofman et al., 1991; Ritchie and Kildea, 1995; Fratiglioni et al., 1999; Lobo et al., 2000) are very similar. Both the prevalence and incidence of dementia are very low before the age of 60, but they increase exponentially with increasing age. The estimated prevalence rates are

approximately 1 % in individuals aged 60–64 years, which increase up to 45 % in the most advanced ages of 95 and above (Fratiglioni and Rocca, 2001).

The prevalence and incidence of AD present the same pattern as dementia in general. After the age of 65 years, the prevalence and incidence of AD approximately doubles every five years. Both prevalence and incidence of VaD has been reported to increase with age, but the increase seems less steep for VaD than for AD (Fratiglioni and Rocca, 2001).

As the population ages, the prevalence of AD has been increasing, and it is becoming an increasingly enormous public health problem. It has been estimated that in the next 50 years, the prevalence of AD will quadruplicate (Brookmeyer et al., 1998). From the same projections, it has been proposed that delaying the onset of the disease by five years would decrease the estimated prevalence by 50 %. Thus, preventive interventions are urgently needed, and these would have a major impact on the future burden of AD.

2.3.2 Risk and protective factors

As suggested above, the exact pathogenic mechanisms of AD remain to be discovered. Various risk and protective factors for AD have been proposed, although there is no unequivocal agreement of their relative importance. The main proposed risk and protective factors from the literature are summarised in tables 1 and 2. Proposed risk factors for AD are divided into established, possible and hypothesised, taking into account the consistency of the findings across different studies, the design of the studies reporting the association, and the biological plausibility.

Clinically, AD can be described as familial and sporadic, early-onset (generally before age 65) and late-onset (after 65), with early-onset predominantly seen in familial cases and late-onset in both familial and sporadic cases. For early-onset familial AD, more than 100 extremely rare and highly penetrant mutations have been described in three genes: amyloid precursor protein (APP), and presenilin 1 (PS-1) and 2 (PS-2) (Tanzi and Bertman, 2001). The first AD gene identified was on chromosome 21, which codes APP. This chromosome was targeted because all individuals with Down's syndrome inherit an extra copy of it and will usually develop the neuropathology of AD by their fourth decade (Mann and Esiri,

1989). However, the mutations in this gene were rarely reported, even in early-onset AD populations and the search for additional genes continued. To date, two other rare but causative genes have been identified; PS-1 on chromosome 14 and PS-2 on chromosome 1.

Familial history of AD is one of the most consistent risk factors for AD, and about 30 % of AD cases have a positive family history (van Duijn et al., 1991). Mutations in APP, PS-1, and PS-2 and the susceptibility gene ApoE cannot entirely account for the observed familial aggregation of the disease, suggesting that additional susceptibility genes exist (Li et al., 1996; Hiltunen et al., 2001; Tanzi and Bertram, 2001). In recent years, significant efforts have been made to find other susceptibility genes, and some potential candidate genes and chromosomes (e.g. 12, 10, 9) have been reported (Hiltunen et al., 2001; Tanzi and Bertram, 2001), but their role in AD still needs to be elucidated.

Familial clustering, however, need not be genetic in origin. Studies in twins have shown that even in concordant twins, the time of disease onset may vary by a decade or more, suggesting that clinical expression may be dependent on shared environmental factors (Chandra and Pandav, 1998, Poirier et al., 1999). Furthermore, because the majority of AD cases do not have any family history, environmental factors can be concluded to have an important role in the disease.

Advanced age is an established risk factor for AD (Fratiglioni and Rocca, 2001). With increasing life expectancies, the obvious question becomes, "If we live long enough, will we all become demented?" Although this question cannot be definitely resolved, accumulating evidence suggest that not all of us will. Recent studies reveal little or no cognitive decline in a great proportion of elderly individuals. The acceleration of incidence rates for AD is also reported to slow down in the very old age groups. Thus, AD has been considered to be age-related rather than ageing-related (Ritchie and Kildea, 1995).

For many years, depression and head trauma have been suggested to be risk factors for AD, and they have been studied in several studies. In spite of all this effort, these possible associations remain highly controversial (Jorm 2000; Fratiglioni and Rocca 2001). Low education has also been proposed as a risk factors for AD, but also here results of different studies are inconsistent and the interpretations are controversial (Fratiglioni and Rocca

2001). Earlier case-control studies have found a negative association between smoking and AD, suggesting that smoking could be a protective factors for AD. These results have later been viewed as potentially biased, and recent longitudinal studies have suggested that smoking may result in a modestly increased risk of AD (Fratiglioni and Wang, 2000 a; Kukull, 2001). There are only few studies concerning alcohol consumption and AD, and the results are again inconsistent. Recent studies have suggested a J-shaped relation, that is, alcoholism may be a risk factor, but a moderate alcohol intake may be protective against dementia (Fratiglioni and Rocca, 2001; Ruitenberg et al., 2002). Recently, reduced intellectual and physical activities at midlife have been associated with an increased risk of late-life AD (Friedland et al., 2001). It has also been reported that rich social network and leisure activities may decrease the risk of dementia (Fratiglioni et al., 2000 b; Wang et al., 2002). High homocysteine level, a cardiovascular risk factor, has also been suggested to be a risk factor for AD (Seshadri et al., 2002). Some hypothesised risk factors and possible protective factors for AD are summarised in table 2 (for review see Fratiglioni and Rocca, 2001; McDowell, 2001).

The current data concerning the role of these proposed risk and protective factors of AD are largely controversial. Accordingly, few generally accepted modifiable risk factors for AD have been identified. Over the last decade, epidemiological evidence has been accumulating that vascular risk factors and indicators of vascular disease may be associated with AD. In particular, hypertension and hypercholesterolemia have regained a considerable amount of attention recently because they may represent common and potentially modifiable risk factors for AD. This thesis will focus on modifiable biological and environmental risk factors versus genetic predisposition for AD with a special interest in future preventive possibilities of the disease. In particular, the following factors will be considered: midlife BP, midlife serum cholesterol levels, ERT, and the ApoE polymorphism.

Table 1. Summary of main proposed risk factors for Alzheimer's disease.

Risk factor	Comments
Established risk factors:	**Strong evidence:**
Advanced age	Numerous studies with consistent results.
Familial aggregation	Numerous studies with consistent results. Both genetic and environmental factors may explain the association.
Down syndrome	Consistent results, biological plausibility; an extra copy of chromosome 21 where the gene for amyloid precursor protein is located.
ApoE ε4 allele	Numerous studies with consistent results. Some evidence of biological mechanisms.
Possible risk factors:	**Some evidence:**
Depression	Many studies with inconsistent results. Risk factor or early manifestation of AD?
Head trauma	Many studies, still inconsistent results. Evidence hampered by recall bias. Biological mechanisms have been postulated.
Female gender	Inconsistent results across comparable studies. Controversial interpretations.
Low education	Several studies but inconsistent results and interpretations. Higher education associated with greater neuronal reserve capacity, thus deferring the onset of the illness? Lower education associated with other risk factors (e.g. vascular) related to AD?
Smoking	Cross-sectional studies: a protective factor for AD, selective survival? Few recent longitudinal studies: a risk factor for AD. Same role as other vascular risk factors?
Alcohol consumption	Few studies, inconsistent results, J-shaped relation suggested.
High blood pressure	Only few cohort studies, inconsistent results*.
High serum cholesterol levels	Only few cohort studies, inconsistent results *.
History of vascular disorder **	Quite consistent results across comparable studies*.
Diabetes mellitus	Some evidence, via vascular mechanisms?
Elevated homocysteine levels	Recent finding, few studies. Controversial interpretations, an independent risk factor AD?
Hypothesised risk factors:	**Limited evidence:**
Occupational exposure, advanced parental age, aluminium, vitamin B12 and folate deficiency, hypothyreodism, sleep apnea etc.	Controversial results and interpretations.

Evidence for each putative factor is summarised taking into account the consistency of the finding across different studies, the design of the studies reporting the association, and the biological plausibility.
*Controversial interpretations and mechanisms unclear; additive mechanisms between vascular and degenerative lesions or direct effect of vascular factors on degenerative lesions?
** Including atherosclerosis, cardiovascular and cerebrovascular disease.

Table 2. Summary of main proposed protective factors for Alzheimer's disease.

Protective factor	Comments
ApoE ε2 allele	Quite consistent results.
Rich social network and leisure activities	Recent finding, few studies. Controversial interpretations.
ERT	Several studies, inconsistent results. Evidence hampered by methodological problems. Good biological plausibility.
Anti-hypertensive drugs	Some evidence, good biological plausibility.
Statins	Some evidence, good biological plausibility.
NSAIDs	Some evidence, good biological plausibility.

Evidence for each putative factor is summarised taking into account the consistency of the finding across different studies, the design of the studies reporting the association, and the biological plausibility.
Abbreviations: ApoE = apolipoprotein E, ERT = estrogen replacement therapy, NSAID = nonsteroidal anti-inflammatory drugs.

2.4 Hypertension and Alzheimer's disease

2.4.1 Hypertension-related changes

Hypertension is a well-recognised as a risk factor for stroke and coronary heart disease (Strandgaard and Paulson, 1994; Kannel, 1996). There is also clear evidence that stroke increases the risk of VaD (Kokmen et al., 1996). High BP is also directly considered to be an important risk factor for VaD (Roman, 1987; Standgaard and Paulson, 1994), as supported by cross-sectional (Prince et al., 1994; Lindsay et al., 1997) and also by some longitudinal studies (Yoshitake et al., 1995; Skoog et al., 1996; Launer et al., 2000).

Until recently, hypertension has rarely been considered as a risk factor for AD. In fact, stroke and severe vascular diseases are generally considered as exclusion criteria for the clinical diagnosis of AD. However, during the last decade, evidence has accumulated suggesting that hypertension and AD might be more closely related than has been traditionally considered. There is evidence that stroke and AD occur in the same patients more frequently than would be anticipated by chance (Pasquier et al., 1998). Interestingly, it has been reported that fewer neuropathological lesions of AD resulted in dementia in those individuals with lacunar infarcts, indicating that brain infarction may influence the clinical expression of AD (Snowdon et al., 1997). Besides for major strokes, hypertension is a risk factor for white

matter lesions (WML) (de Leeuw et al., 2002), which are commonly found in dementia and AD at old age (Skoog et al., 1996; Breteler et al., 1998). Chronic hypertension can also lead to capillary damage, and the cerebral capillary ultrastructure has been observed to be damaged in AD (Farkas and Luiten, 2000). Interestingly, Sparks et al. (1995), found in an obduction serie an increased incidence of NFTs and senile plaques in people with a history of hypertension, suggesting a link between hypertension and the formation of these neuropathological changes. Later, this hypothesis has gained support by another study reporting an increased amount of NPs and NFTs, and increased brain atrophy, in individuals with elevated BP at midlife (Petrovitch et al., 2000).

2.4.2 Hypertension and AD in epidemiological studies

Several cross-sectional studies have evaluated the association between BP and AD, but with conflicting results. Many of these studies have in fact found that BP levels in patients with AD are lower than those without dementia (Kokmen et al., 1991; Elmståhl et al., 1992; Kilander et al., 1993; Landin et al., 1993; Wang et al., 1994; Guo et al., 1996; Hogan et al., 1997). Some relatively short follow-up studies have investigated the relation between BP and AD, but yielded also somewhat contradictory or negative results. The main longitudinal studies on the association between BP and AD are summarised in table 3.

A follow-up study over seven years conducted in a Japanese population among individuals aged 65 and older observed an increased risk of VaD as the level of systolic BP increased, but no significant association between BP and AD was found (Yoshitake et al., 1995). A three-year follow-up study from the Kunsgholmen Project among individuals aged 75 or older found that baseline high systolic BP ($\geq$ 180 mmHg) was related to an increased risk of dementia (Guo et al., 2001). However, the association was not statistically significant when all covariables were considered in the model (table 3). The study found no association between baseline low BP and increased incidence of dementia, but interestingly, a reduction of BP $\geq$ 5 mmHg over the three-years of follow-up was found to be associated with an increased risk of dementia (Guo et al., 2001). A recent study among individulas aged 65 years and older found no association between a history of hypertension and AD during a seven-year follow-up period (Posner et al., 2002).

A conceptual limitation especially in cross-sectional studies, but also in short-term longitudinal study settings is that they cannot prove a causal relationship, but only infer an association. From a causal point of view, stronger evidence of the association between these vascular risk factors and AD is provided by long-term prospective studies. Until recently, such evidence has been lacking. During the last six years, this gap has been filled by a few population-based studies that have follow-up times ranging from around 10 to 25 years.

The first long-term longitudinal study examining the relation between BP and the subsequent development of dementia was conducted as part of the Longitudinal Population Study of 70-year-old individuals in Gothenburg, Sweden (Skoog et al., 1996). A sample of 382 individuals was followed for up to 15 years. It was found that high BP at the age of 70 years increased the risk of AD 9–15 years later. At the time of the first examination, both systolic and diastolic BP were higher in the subjects who eventually developed dementia, but the risk for subsequent AD was only significant for diastolic BP.

The second study, the Honolulu-Asia Aging Study examined the association between midlife (mean age 53 years) BP with late-life AD in a cohort of 3703 Japanese-American male subjects followed for 25 years (Launer et al., 2000). It was found that elevated diastolic BP in midlife increased the risk of AD. It is noteworthy that this relation was evident only in those individuals who had never been treated with antihypertensive drugs, and there was no association between AD and BP in treated hypertensive men.

In contrast to these two longitudinal studies, a recent longitudinal study in Boston of 634 participants aged 65 years or older, found little association between BP levels during 15 years of observation and risk of AD (Morris MC et al., 2001). There was no evidence of an increased risk of AD among persons with high BP thirteen years before dementia diagnosis, an inverse association for BP measured four years before diagnosis, and no effect of AD on BP measures two years after the diagnosis.

In conclusion, the relation between BP and AD is still controversial. Representative, population-based studies with long follow-up times are needed to elucidate this issue further.

Table 3. Main longitudinal studies on the association between blood pressure and AD.

Author	Study Population	Follow-up time	Outcome,	Results *
Yoshitake et al., 1995	Japanese population, mean age 73 years for men, 74 years for women, n = 828	7 years	Dementia (n = 103), VaD (n = 50), AD (n = 42)	Every 1 SD increase in baseline SBP: RR = 1.6 (1.2-2.2) for VaD, RR = 1.0 (0.75-1.4) for AD
Skoog et al., 1996	Longitudinal Population Study of 70-year-olds in Göteborg, Sweden, n = 382	15 years	Dementia (n = 18), AD (n = 10), VaD (n = 7)	Elevated DBP at age 70 increased the risk of AD at age 79-85; Elevated DBP at age 75 increased the risk of VaD at age 79-85
Launer et al., 2000	Honolulu-Asia Aging Study, Japanese-American men, mean age 53 years, n = 3703	25 years	Dementia (n = 197), AD (n = 118), VaD (n = 79)	Baseline DBP ≥ 95 mmHg: OR = 4.5 (1.5-13.1), DBP 90 –94 mmHg: OR = 3.5 (1.3-9.5) for AD among men never treated with antihypertensive medications. Baseline SBP ≥ 160 mmHg: OR = 4.0 (1.5-10.0) for total dementia, OR = 11.8 (3.5-39.5) for VaD
Guo et al., 2001	Kungsholmen Project, age 75+ (mean age not stated), n = 1270	3 years	Dementia (n = 218)	Baseline SBP ≥ 180 mmHg: age and gender adjusted RR = 1.6 (1.1-2.5), after further adjustments for education, vascular disease, and antihypertensive medication, RR = 1.4 (0.9-2.2)
Morris MC et al., 2001	East Boston Established Populations for Epidemiologic Studies of the Elderly, age 65+, n = 634, only a subgroup (n=378) had BP measured 13 years before the diagnosis	15 years	AD (n = 99)	No association between SBP measured 13 years before AD diagnosis: OR = 1.0 / 10 mmHg increase in SBP (0.80–1.32); an inverse association with SBP measured 4 years before: OR = 0.80 / 10 mmHg (0.72-0.95)
Ruitenberg et al., 2001a	Pooled dataset based on Rotterdam study (n = 6668, age 55+) and Gothenburg H-70 Study (n = 317, age 85 years)	2.1 years	Dementia (n = 196)	The risk of dementia decreased with increasing BP in persons who used antihypertensive medications; per 10 mmHg increase in SBP: RR = 0.93 (0.88-0.99); per 10 mmHg DBP: RR 0.89 (0.79-1.00)
Posner et al., 2002	Washington Heights-Inwood Columbia Aging Project, mean age 79 years for AD group, 75 years for controls, n = 1259	7 years	AD (n = 157) VaD (n = 56)	History of hypertension: RR = 0.9 (0.7-1.3) for AD, RR = 1.8 (1.0-3.2) for VaD (≅ similar results by direct measurement of BP)

Abbreviations: DBP = diastolic blood pressure, OR = odds ratio, RR = risk ratio, SBP = systolic blood pressure, SD = standard deviation. * Results are given as ORs or RRs with 95% confidence intervals. Age indicates the age at baseline.

2.4.3 Hypertension and cognitive impairment in epidemiological studies

"*Impairment of cerebral functions equivalent to that seen in patients with surgical removal of both frontal lobes may occur early in the course of essential hypertension without neurological signs*" was already reported more than half a century ago (Apter et al., 1951). Thus, the view that there may be an association between hypertension and cognitive impairment is not novel. The first study that addressed this issue more specifically was published in 1971 by Wilkie and Eisdorfer. A group of 202 volunteers was evaluated using the Wechsler Adult Intelligence Scale (WAIS) at approximately ten-year intervals. However, only 87 individuals were examined at the last examination. The study reported a correlation between hypertension (elevated diastolic BP) and intellectual loss over the follow-up period.

After that study, several cross-sectional studies have investigated the relationship between BP and late-life cognitive function, but results obtained from these studies have been controversial (Viitanen et al., 1997). Recently, some longitudinal studies have investigated the relationship between BP earlier in life and late-life cognitive function in non-demented subjects (table 4). Some studies with a relatively short follow-up time including very old patients have reported a J-curve profile with an increase in cognitive impairment in subjects with low BP (Guo et al., 1997; Okumiya et al., 1997). From the causal point of view, the strongest evidence concerning the association between BP and cognitive impairment can be considered to come from the studies with a relatively long follow-up time. Interestingly, many of the long-term prospective studies have suggested that chronic hypertension may alter cognitive functioning. They have described an association between elevated systolic BP (the Honolulu-Asia Aging Study) (Launer et al., 1995), elevated diastolic BP (the Uppsala Study) (Kilander et al., 1998), both elevated systolic and diastolic BP (the Framingham Study) (Elias et al., 1993), or hypertension in general (National Heart, Lung, and Blood Institute Twin Study) (Carmelli et al., 1998) at midlife and impaired cognitive performance in late-life. On the other hand, a recent longitudinal population-based study indicated a complex relationship between elevated BP and cognitive decline with the results varying according to which test was used to measure cognition, reflecting a rather minor association between BP and cognition (Glynn et al., 1999).

In conclusion, results from the published studies about the association between hypertension and cognitive impairment remain inconclusive. One problem with these earlier studies is that the outcome has not been uniformly defined across studies. Instead, cognitive impairment has been defined by performance on a variety of neuropsychological tests, and not by any clinical or diagnostic concepts. This limits the interpretation and comparison of the results. Recently, one longitudinal study on BP and MCI was published, reporting an association between elevated diastolic BP at midlife and increased risk for MCI (DeCarli et al., 2001). The diagnosis of MCI was based on delayed free recall performance on the California Verbal Learning Test. There appear to be no studies specifically investigating the relationship between midlife BP and the development of MCI applying the proposed diagnostic criteria (Petersen et al., 2001 a; 2001 b).

Table 4. Main longitudinal studies on the association between hypertension and cognition.

Author	Study population	Follow-up time	Cognitive assessment	Predictor of cognitive impairment
Wilkie et al., 1971	A sample of volunteers, n = 202 (87 at last evaluation), mean age 79 years	10 years	WAIS	High DBP at baseline, but no correlation for borderline hypertension between age 60–69
Elias et al., 1993	Framingham Study, n = 1702, age 55 - 88 years at baseline	12 -14 years	Kaplan Albert Battery	Elevated DBP and SBP at baseline
Launer et al., 1995	Honolulu-Asia Aging Study, n = 3735 males of Japanese descent, mean age 78 years	25 years	CASI	Elevated SBP at baseline
Guo et al., 1997	Kungsholmen Project, n = 1736, age 75 - 101 years at baseline	3.4 years	MMSE	Baseline low SBP (< 130 mmHg) in untreated subjects, and high SBP (> 180 mmHg) in patients treated with antihypertensive drugs
Okumiya et al., 1997	n = 155, mean age 78 years	3 years	MMSE	J-curve relation between BP and decline on MMSE score
Kilander et al., 1998	Uppsala Study, n = 999 men, mean age 72 years	20 years	MMSE, Trail Making Test	Elevated DBP at baseline
Carmelli et al., 1998	National Health, Lung, and Blood Institute Twin Study, n = 410 males, mean age 73 years	25 years	MMSE, DSS Test, BVRT	BP > 140/90 mmHg or use of antihypertensive medication at baseline were associated with a significantly greater decline on the DSS test
Glynn et al., 1999	Established Populations for the Epidemiologic Study of the Elderly, Boston, n = 2068, age 65 - 102 at baseline	9 - 15 years	SPMSQ, EBMT	BP had rather minor effect on cognitive functions. Elevated SBP 9 years earlier was associated with an increased error rate on SPMSQ. DBP 9 ears before, and baseline SBP had a U-shaped association with errors on SPMSQ
DeCarli et al., 2001	National Health, Lung, and Blood Institute Twin Study n = 369, mean age 73 years	25 years	CVLT	Elevated DBP at baseline

Abbreviations: BVRT = Benton Visual Retention Test, CASI = Cognitive Abilities Screening Instrument, California Verbal Learning Test, DBP = Diastolic blood pressure, DSS = Digit Symbol Substitution, EBMT = East Boston Memory Test, MMSE = Mini-Mental State Examination, SBP = systolic blood pressure, SPMSQ = Short Portable Mental Status Questionnaire, WAIS = Wechsler Adult Intelligence Scale.
Age indicates the age at the time of cognitive testing. Age-range at baseline is used if mean age was not stated in the study.

2.4.4 Antihypertensive agents and AD

In addition to the recent epidemiological studies, the second line of evidence that can be adopted when discussing the relation between hypertension and the risk of AD comes from clinicoepidemiological studies evaluating the association between the usage of antihypertensive medications and the risk of AD.

The issue of antihypertensive medication and the incidence of dementia has been highlighted in only a few randomised placebo-controlled hypertension trials. The Systolic Hypertension in the Elderly Program (SHEP, 1991) involved 4736 subjects aged 60 years and older with isolated systolic hypertension. The diuretic chlorthalidone was used as the primary drug in the active treatment group (n = 2365). After an average follow-up period of five years, the incidence of dementia did not differ between the active treatment (1.6 %) and placebo groups (1.9 %). The cognitive sub-study of the Medical Research Council trial (Prince et al., 1996) included 2584 subjects aged 65-74 years with hypertension randomised to a diuretic, β-blocker, or placebo. No significant difference in psychometric tests was detected between the active treatment and placebo groups covering a period of approximately four and a half years.

Data from the Systolic Hypertension in European (Syst-Eur) trial (Forette et al., 1998) is the first and also the only randomised double-blind placebo-controlled study to date to show that treatment of hypertension may reduce the risk of dementia and, more specifically, that of AD. The study included 2418 subjects 60 years or older with isolated systolic hypertension. Compared with placebo (n = 1180), active treatment (n = 1238) of isolated systolic hypertension with nitrendipine, a calcium-channel blocker, was found to half the incidence of dementia from 7.7 to 3.8 cases per 1000 patient-years (21 vs. 11 patients). The primary hypothesis of that study was that the reduction in BP would protect against vascular dementia, and thus, the decreased incidence of AD was described as unexpected by the authors. The sample size in the study was large, but the follow-up time was relatively short, with a median of two years, and the number of demented subjects was quite small. The publication of this study sparked a debate on whether the effect was due to the reduction in hypertension or the ability of calcium channel blockers to reduce calcium-mediated neural damage but, irrespective of the mechanism, the effect was present.

The effects of antihypertensive treatment on the incidence of dementia have also been evaluated in some observational studies. In the Kungsholmen Project (Guo et al., 1999), Stockholm, Sweden, 1301 subjects aged 75 years and older without dementia at baseline were followed for an average of three years. Subjects taking diuretics at baseline had a significantly reduced incidence of dementia. According to the authors, AD accounted for more than 70 % of incident dementia cases, but AD and vascular dementia were not specifically differentiated in that study. Moreover, the association of antihypertensive drug use and the risk of dementia was recently investigated in the context of the Rotterdam Study (in't Veld et al., 2001). This observational study, including 7046 elderly individuals aged 55 years or older, free of dementia at baseline, reported that after a mean follow-up time of 2.2 years, antihypertensive medication protected against vascular dementia, but no significant protective effect was found for AD. The authors concluded that while dementia may be prevented by antihypertensive treatment in hypertensive patients, larger studies with longer follow-up periods are needed to confirm the relation between BP changes and the risk of AD.

2.5 Hypercholesterolemia and Alzheimer's disease

2.5.1 Hypercholesterolemia-related changes

Besides hypertension, hypercholesterolemia is a known risk factor for atherosclerosis and coronary artery disease. Both of these conditions have been associated with AD (Sparks et al., 1995; Sparks et al., 1997; Hofman et al., 1997), suggesting an association between hypercholesterolemia and AD. It is known that longstanding hypercholesterolemia may lead to intima thickening and weakening of endothelial functions in cerebrovascular arterioles and capillaries (Levine et al., 1995). These changes may impair brain metabolism, and eventually lead to cognitive failure and dementia. The ApoE ε4 allele, the most important genetic risk factor for AD, is associated both with elevated serum cholesterol, which has also raised the question of whether cholesterol levels have some role in the development of AD. Jarvik et al. (1995) reported in their case-control study that the relationship between ApoE genotype and AD was dependent on total cholesterol suggesting that cholesterol may be involved in the pathology of AD.

In addition to this evidence suggesting an association between cholesterol and AD, data from experimental studies have pointed in the same direction. The first finding that suggested a link between cholesterol and AD etiology at the molecular level was obtained from rabbits, which do not normally develop Aβ deposits. Animals fed with cholesterol were found to rapidly accumulate Aβ in the brain (Sparks et al., 1994). Later, it has been reported that high cholesterol uptake increase Aβ deposition in transgenic mice (Refolo et al., 2000). Thus, it has been suggested that cholesterol may affect Aβ production in a similar way to the genetic mutations in APP, PS-1 and PS-2.

The possible biological mechanisms of how cholesterol would affect Aβ and APP have gained great interest lately, and many interesting experimental studies have been published in recent years. Bodovitz and Klein (1996) studied the effects of elevated cholesterol levels on the processing of APP *in vitro* and concluded that high cholesterol consentrations could reduce the secretion of soluble APP (APPα). This association has been demonstrated also by more recent *in vitro* studies (Racchi et al., 1997; Galbete et al., 2000). Furthermore, depletion of intraneuronal cholesterol has been recently reported to inhibit Aβ production *in vitro* (primary neurons) (Simons et al., 1998) and *in vivo* (in guinea pigs) (Fassbender et al., 2001). These findings suggest that increased cholesterol levels could accelerate the production of Aβ, the accumulation of Aβ in plaques, and the development of AD.

2.5.2 Hypercholesterolemia and AD in epidemiological studies

Results of the published studies on cholesterol levels in patients with AD have been conflicting. A recent meta-analysis of ten studies reported that reported patients with AD had somewhat lower levels of plasma total cholesterol than controls (difference 0.17 mmol/l) (Knittweis et al., 2000). There are some prospective studies with relatively short follow-up times yielding also negative or conflicting results on the association between cholesterol and AD (table 5). The study of Kuusisto et al. (1997) reported that low total cholesterol levels 3.5 years before diagnosis and at the time of diagnosis were associated with AD. One study with a mean follow-up time of 2.5 years, reported a small but statistically significant decrease in total cholesterol levels in individuals who developed AD (Romas et al., 1999).

Consistent with these results, the Rotterdam study reported lower plasma total cholesterol levels in demented patients (Slooter et al., 1999). In the extended follow-up data (mean follow-up time 5.8 years) of the study cohort, mean total cholesterol level was significantly lower in prevalent as compared to incident dementia, but overall, total cholesterol at baseline was not related to dementia or AD at follow-up (Slooter et al., 2000). Interestingly, however, in the Rotterdam Study, high dietary baseline of total fat, saturated fat, and cholesterol were found to be related to an increased risk of dementia (RR = 2.4, 95 % CI 1.1–5.2, RR = 1.9, 95 % CI 0.9–4.0, RR = 1.7, 95 % CI 0.9–3.2, respectively) during the two-year follow-up (Kalmijn et al., 1997). Fish consumption on the other hand, an important source of n-3 polyunsaturated fatty acids, was inversely related to incident dementia (RR = 0.4, 95 % CI 0.2-0.9), and in particular to AD (RR = 0.3, 95 % CI 0.1–0.9).

In one prospective community-based study, a total of 1111 non-demented participants were followed for an average of 2.1 years (Moroney et al., 1999). Levels of low-density lipoprotein (LDL) cholesterol were significantly associated with an increased risk of dementia with stroke, but not with the development of AD.

The only long-term prospective study on the association between cholesterol and AD is the Finnish cohort of the Seven Countries Study (Notkola et al., 1998), conducted in 444 elderly males. The study reported that men with AD had had elevated serum cholesterol levels 15–25 years before the onset of AD. In men who subsequently developed AD, the cholesterol levels decreased before the clinical manifestation of AD, and the decline was more rapid than among men who did not develop dementia. As a result, cholesterol levels of AD cases were lower at the time of diagnosis than those of the non-demented.

In conclusion, the recent studies on the association between cholesterol and AD have been mainly cross-sectional or relatively short follow-up studies and, again, have yielded conflicting results. Consequently, long-term prospective studies might shed light on the issue.

Table 5. Main longitudinal studies on the association of cholesterol with Alzheimer's disease.

Author	Study Population	Follow-up time	Outcome	Results *
Kuusiso et al., 1997	Population-based study in Kuopio, Finland, n = 980, age 69–78 years	3.5 years	AD (n = 46)	Increasing total cholesterol at baseline; OR = 0.67 (0.50-0.87) for AD, at the time of diagnosis; OR = 0.69 (0.52 – 0.92)
Notkola et al., 1998	Finnish male Cohort of the Seven Countries Study, n = 444, age 77–89 years	15–25 years	AD (n = 27)	High total cholesterol at baseline; OR = 3.1 (1.2–8.5) for AD
Romas et al., 1999	Community- based study of white, African American, and Caribbean Hispanic elderly in New York City, n = 1238, mean age 76 years	2.5 years	Prevalent AD (n =178), Incident AD (n = 129)	Low total cholesterol at baseline; OR = 1.3 (0.8–2.1) for prevalent AD, OR = 1.6 (1.0–2.7) for incident AD
Moroney et al., 1999	Community-based study in Manhattan, New York City, n = 1111, mean age 75 years at baseline	2.1 years	AD (n = 225), VaD (n = 61)	High LDL cholesterol at baseline; RR = 3.1 (1.5–6.1) for dementia with stroke, RR = 0.77 (0.51–1.15) for AD
Slooter et al., 2000	Rotterdam Study, n = 6435, mean age not stated	5.8 years	Dementia (n = 395)	Total cholesterol at baseline; RR = 0.97 (0.89–1.06) for incident dementia and RR = 0.99 (0.89–1.10) for AD

Abbreviations: ApoE = Apolipoprotein E, AD = Alzheimer's disease, BP = blood pressure, VaD = vascular dementia.
*Results are given as adjusted odds ratios (OR) or risk ratios (RR) with 95% confidence intervals. Age indicates the age at the time of diagnoses unless otherwise indicated.

2.5.3 Hypercholesterolemia and cognitive impairment in epidemiological studies

The findings of the cross-sectional studies that have investigated the relationship between serum lipoprotein levels and cognitive impairment are again conflicting (Breteler et al., 1998). Furthermore, there have been very few longitudinal studies examining the relationship between midlife serum cholesterol levels and late-life cognitive impairment. Recently, an observational study of 1307 postmenopausal women with coronary heart disease enrolled in the Heart and Estrogen/progestin Replacement Study evaluated whether serum lipoprotein levels, the four-year change in these levels, and the use of statin drugs were associated with cognition using the Modified MMSE (Yaffe et al., 2002). The study revealed that women in the highest LDL cholesterol quartile had an increased risk of cognitive impairment (OR = 1.8,

95 % CI 1.0-3.0). Compared with non-users, statin users had higher mean Modified MMSE scores and a trend for a lower risk of cognitive impairment (OR = 0.7, 95 % CI 0.4-1.1). So far, there appear to be no studies on the association between cholesterol and MCI.

2.5.4 Lipid-lowering agents and AD

Two recent retrospective clinical studies have reported significantly reduced rates of dementia in subjects who had used statins as cholesterol reducing drugs. A cross-sectional analysis including 57 104 patients aged 60 years and older from databases at three different hospitals, reported 60 to 73 % reduced prevalence of AD in the cohort taking statins compared to the total population (Wolozin et al., 2000). The protective effects were found for lovastatin and pravastatin but not for simvastatin or other medications typically used in the treatment of hypertension and cardiovascular disease. One potential reason for the negative association for simvastatin may lie in differences in physicians' prescribing patterns; simvastatin is a slightly newer drug than the two other statins, and the authors later reported that prescription of simvastatin in their institute had begun more recently than the other two statins (Wolozin et al., 2001).

Another study derived data from the General Practice Research Database in the United Kingdom, consisting of a base population of 60 901 individuals, aged 50 years and older. From these data, they used a nested case-control study design with 284 cases with dementia and 1080 controls (Jick et al., 2000). The study did not distinguish between AD and other forms of dementia. The risk of dementia was found to be up to 70 % lower in individuals using statins than those who did not have hyperlipidemia or those with untreated hyperlipidemia. It is noteworthy that individuals with hyperlipidemia receiving lipid-lowering drugs other than statins did not have a reduced risk of dementia. The protective effect was similar for all individual statins, including simvastatin.

While these results suggest a protective effect of statins on dementia, they are also suffer from some limitations. The study designs used are susceptible to indication bias, and information on important potential confounding factors, such as education, were not available. In addition to the two retrospective study discussed above, a recent secondary analysis of the Canadian Study of Health and Aging (Rockwood et al., 2002), a population-based survey of Canadians

65 years and older, examined the issue using a cohort design (n = 2305) to assess the possibility of indication bias, and a case-control setting to evaluate whether the use of lipid-lowering agents were associated with dementia (492 incident dementia cases and 823 controls). To minimise indication bias, only incident dementia cases were used (mean follow-up time four to five years). No indication bias was found, and the use of statins and other lipid-lowering agents was associated with lower risk of dementia, and specifically of AD in individuals younger than 80 years even after adjustment for sex, education, and self-rated health. Thus, this study supports the observation of a protective association between statins and dementia, and suggests that it might also be extended to other lipid-lowering agents.

2.6 Existing vascular disease and Alzheimer's disease

Until recently, clinical vascular disease has seldom been studied in relation to AD. Because of the diagnostic criteria for AD, patients with clinical vascular disease are less likely to be diagnosed with AD (Breteler 2000). Some case-control studies showed cardiovascular disease (Tresch et al., 1985) and diabetes mellitus (Bucht et al., 1983; Landin et al., 1993; Mortel et al., 1993; Nielson et al., 1996; Wolf-Klein et al., 1998; Tariot et al., 1999) to be positively associated with vascular dementia, but inversely with AD. However, these studies had a number of methodological limitations. For example, many studies were based on selective patients and controls, the presence of heart disease and diabetes mellitus was assessed from medical records and not actually screened for, and subjects with any indication of vascular disease were rigorously excluded from the patient series (Breteler, 2000).

More recent studies have reported an association between various vascular diseases and AD. A positive relation between history of myocardial infarction (MI) among women developing AD has been described (Aronson et al., 1990). In addition, coronary artery disease at autopsy has been found to be associated with a significant increase in the cortical senile plaques in the brain (Sparks et al., 1995). In the Rotterdam Study, atrial fibrillation was significantly more frequent among individuals with dementia, particularly AD. The associations were stronger in women than in men (age-adjusted OR = 3.1, 95 % CI 1.7–5.5 vs. OR = 1.3, 95 % CI 0.5–4.1, respectively) (Ott et al., 1996). Many recent studies, both cross-sectional (Ott et al., 1996; Peila et al., 2002) and longitudinal (Leibson et al., 1997; Brayne et al., 1998; Ott et al., 1999), have shown a positive association between diabetes and AD. However, there are also

longitudinal studies finding only a marginally significant association (Yoshitake et al., 1995; Luchsinger et al., 2001) or no association (Curb et al., 1999) between diabetes and AD.

The study of Kuusisto et al. (1997) reported that features of insulin resistance syndrome 3.5 years before the diagnosis and at the time of diagnosis were associated with AD. In the Honolulu-Asia Aging Study, a higher cardiovascular metabolic risk factor burden (random postload glucose, diastolic and systolic BP, body mass index (BMI), subscapular skin fold thickness, random triglyserides, and total cholesterol) in middle age increased the risk of dementia 25 years later (Kalmijin et al., 2000). Clustering of these cardiovascular metabolic risk factors was specifically associated with VaD, but not with AD.

In a cross-sectional analysis of the Rotterdam Study, indicators of atherosclerosis of the carotid artery (wall thickness and plaques as measured by ultrasonography) and presence of atherosclerosis of the large vessels of the legs (assessed by the ratio of the ankle-to-brachial systolic BP) were associated with AD, and the prevalence of AD increased with the degree of atherosclerosis (Hofman et al., 1997). However, an autopsy study found no association between cardiovascular index calculated at autopsy (the status of the heart, generalised arteriosclerosis, and the arteriosclerosis of arteries of circle of Willis) and Alzheimer's lesions in the brain (Alafuzoff et al., 1999).

2.7 Apolipoprotein E and Alzheimer's disease

2.7.1 Structure and function of ApoE

ApoE is a glycoprotein of 299 amino acids with a molecular mass of ~34kDa. ApoE was discovered in the early 1970s as a protein component of triglyceride rich lipoproteins. It immediately became apparent that ApoE was an important determinant in cholesterol metabolism, and ApoE became the focus of intense scientific scrutiny (Utermann et al., 1977; Utermann et al., 1984; Robertson et al., 1985). In the mid-1970s, Utermann and colleagues found that ApoE was polymorphic, exhibiting multiple isoforms as detected by isoelectric focusing. Polymorphism within the human ApoE gene, located on chromosome 19, is the result of three alleles (ε2, ε3, and ε4) at a single gene locus and accounts for the three major ApoE isoforms (E2, E3, E4). ApoE ε3 is the most common allele (77–78 %) in the general

population, while ApoE ε2 is found in 7–8 %, and ApoE ε4 in 14–16 % of individuals, yet frequencies may vary between various geographic and ethnic populations (Mahley and Rall, 2000; Cedazo-Mínguez and Cowburn, 2001). The three isoforms differ from one another in primary structure only by single amino acid substitution at two sites. ApoE3 has cysteine at residue 112 and arginine at residue 158, while ApoE4 has arginine and ApoE2 cysteine at both sites. Yet, these single amino acid substitutions lead to charge differences detectable by isoelectric focusing and affect the three-dimensional structure and lipid binding properties of the isoforms (Beffert et al., 1998; Cedazo-Mínguez and Cowburn, 2001).

ApoE is synthesised and secreted by many tissues, primarily the liver, brain, skin, and tissue macrophages throughout the body. Despite a number of factors being described, the mechanisms governing ApoE synthesis and secretion in brain are not fully understood. The understanding of how ApoE production is regulated in the central nervous system (CNS) remains an important issue that may provide clues about ApoE function in normal and AD brains (Cedazo-Mínguez and Cowburn, 2001).

2.7.2 ApoE and risk of AD

For a long time, the ApoE ε4 allele has been known to be a risk factor for (cardio)vascular disease (Menzel, 1983). Following this connection, the first link between dementia and ApoE ε4 was established in 1989, when an increased ε4 allele frequency in multi-infarct dementia was reported (Shimano et al., 1989). Discovery of the link between ApoE and AD, however, took place apparently in a more incidental manner, as is often the case with great inventions. In 1986, an increase of ApoE synthesis following crush injury of the sciatic nerve was reported, suggesting a role for ApoE in the repair response to nervous tissue injury (Ignatius et al., 1986). Turning more specifically to AD, Namba et al. (1991) reported that ApoE immunoreactivity was associated with amyloid in senile plaques and cerebral vessels, NFTS in AD brains, as well as kuru plaques in brains from patients with Creuztfeldt-Jacob disease. In the same year, genetic linkage analysis suggested a linkage of familial AD to chromosome 19q13.1 - q13.3, i.e., the very region where the gene for ApoE is located (Pericak-Vance et al., 1991). Then, in 1993, when examining the binding properties of ApoE to amyloid in cerebrospinal fluid (CSF), Strittmatter et al. (1993) reported "*an unexpected overrepresentation of the APOE-ε4 allele in late-onset familial Alzheimer's disease compared*

with age-matched, unrelated controls". This finding sparked an enormous body of research. Already by the end of 1993, the ε4 allele was also confirmed as a risk factor for sporadic forms of AD (Poirier et al., 1993) and was found to be related to earlier onset of AD (Corder et al., 1993).

The association between the ApoE ε4 allele and AD has now been confirmed in number of studies worldwide in both sporadic and late-onset familial cases of AD (Farrer et al., 1997). To date, ApoE ε4 is the only genetic risk factor for AD of established general significance. The risk increases in a dose-dependent manner; the risk of AD increases, and the age of onset decreases, with the number of the ε4 alleles. There are, however, some ethnic variations in the association between ApoE ε4 and AD, and only a weak or no relationship between ApoE ε4 and AD has been reported among African-Americans, Hispanics, and Nigerians (Tang et al., 1998; Hendrie et al., 2001). In contrast to the ApoE ε4 allele, ε2 seems to exert a protective effect toward AD.

Although ApoE ε4 is an established risk factor for AD and lowers the age of onset, its effect on the rate of progression of AD is less obvious. Some initial reports suggested that ApoE ε4 could accelerate the progression, but some recent studies have not found an influence on the progression or survival of the disease (Slooter et al., 1999 a; Koivisto et al., 2000). Yet, ApoE ε4 has been reported to be an important predictor of AD among subjects with MCI (Petersen et al., 1995). It was initially reported that brains of AD patients carrying the ε4 allele had an increased number of plaques (Schmechel et al., 1993; Polvikoski et al., 1995; Pirttilä et al., 1997) and NFTs (Ohm et al., 1995; Polvikoski et al., 1995), and more severe cholinergic deficits (Soininen et al., 1995 a; b) than in those without the ε4 allele. However, some subsequent studies have not been able to replicate these findings (Blennow et al., 1996; Morris et al., 1996). Thus, despite the number of publications on ApoE and AD to date, the research has resulted in several controversies, and the exact role of the ApoE in the pathogenesis of AD remains to be established.

The major difference between individuals carrying the ApoE ε4 allele and those carrying APP, PS-1, or PS-2 mutations, is that carriers of the ApoE ε4 allele have a good chance of escaping AD even in old age, as the ε4 allele lowers the age of disease onset rather than causing the

disease. Thus, the ApoE is called a susceptibility gene for AD and it is neither necessary nor sufficient for AD to develop. Mutations in the three genes (APP, PS-1, PS-2) of AD are known to be involved in the molecular pathways of AD and to cause increased Aβ42 production (Tanzi and Bertman, 2001). Unlike the other proteins, ApoE is not known to be directly involved in Aβ generation, although it could be indirectly involved. Perhaps the best indication that ApoE plays a role in Aβ pathology is derived from experiments using ApoE transgenic or knockout mice, as they show altered depositions (Bales et al., 1997). Until now, ApoE is also the only one of these genes known to be involved in lipid pathways. The role of ApoE as a lipid-transport protein suggests that lipids might somehow influence Aβ production. Accumulating data suggests that ApoE has multiple functions in the brain and also that different ApoE alleles possess specific properties. In addition to human studies, data from *in vitro* and *in vivo* studies have shown that ApoE ε4 intensifies many biochemical characteristics of AD, including Aβ deposition, tangle formation, cholinergic signalling, neuronal cell death, oxidative stress, synaptic plasticity, and lipid dysfunction (Cedazo-Mínguez and Cowburn, 2001).

It is important to note that ApoE polymorphism is not only associated with the risk of AD, but also with the risk of other dementias and other neurodegenerative disorders besides vascular risk factors and diseases (reviewed in the next paragraph). ApoE ε4 is associated with a worse outcome after stroke and head injury and faster progression of multiple sclerosis and motor neuron disease (Chapman et al., 2001). The association of ApoE ε4 with other disorders highlights its relevance to brain pathology in more general terms and suggests that it may confer general, non-specific hypersensitivity to brain injury.

2.7.3 ApoE and vascular factors

ApoE has a central role in lipid metabolism, and ApoE ε4 allele is associated with increased serum total and LDL cholesterol levels (Davignon et al., 1998) atherosclerosis, and coronary heart disease (Wilson et al., 1996). Thus, the question about the interrelationships between ApoE and vascular factors in the development of AD is of interest. Earlier cross-sectional or short follow-up studies concerning the putative interrelationship between ApoE, cholesterol, and AD have yielded conflicting results (Jarvik et al., 1995; Romas et al., 1999; Frikke-

Schmidth et al., 2001). Again, it is the long-term prospective studies which may be better in resolving this issue. However, only two such studies exist. The first reported that elevated serum cholesterol was a risk factor for AD independently of ApoE ε4 allele, but the association between ApoE ε4 and AD became weaker after controlling for serum cholesterol (Notkola et al., 1998). Thus, the authors concluded that some of the effects of the ApoE ε4 allele on the risk of AD might be mediated through elevated serum cholesterol levels. On the contrary, another follow-up study suggested that the presence of ApoE ε4 increases the risk of AD independently of its effect on dyslipidemia and atherogenesis (Prince et al., 2000). Thus, it is not known whether the associations between elevated serum cholesterol levels, ApoE polymorphism, and AD are independent or interrelated.

Higher BP values among the ε4 carriers have been described (Uusitupa et al., 1994), but in general, the association between ApoE and BP appears to be vague and most studies have not found any consistent influence of ApoE on BP (de Knijff et al., 1994; Wilson et al., 1994; Prince et al., 2000). However, some studies have reported excess cognitive decline in hypertensive individuals carrying the ApoE ε4 allele (Carmelli, et al., 1998; Haan et al., 1999; Peila et al., 2001). Moreover, atherosclerosis, which can be seen as a consequence of elevated cholesterol or BP, has been studied in relation to AD and ApoE polymorphism, but also with conflicting results. First, at the cross-sectional level of the Rotterdam Study, a strong interaction between ApoE ε4 and atherosclerosis was found for the development of dementia (Hofman et al., 1997), but later, at the incident phase of the same study, the interaction had disappeared (Slooter et al., 1999 b). Interestingly, a recent population-based study found a significant association between the ApoE ε4 allele and AD only among people not using antihypertensive drugs (Guo et al., 2001).

2.8 Gender and hormonal effects and Alzheimer's disease

2.8.1 Does gender make a difference?

Prevalence rates of AD have been reported to be 1.5-3 times greater among women than among men. This is partly because of greater longevity among women; women also survive longer with AD. A higher incidence rate of AD in women compared to men, especially after the age of 85 years has been described in some (Andersen et al., 1999; Fratiglioni et al., 2000 c) but not in all studies (Bachman et al., 1993; Letenneur et al., 1994; Rocca et al., 1998; Ganguli et al., 2000). Recent findings from the large population-based Rotterdam Study reported no difference in the incidence of dementia up to a high age. Only after 90 years of age the incidence of AD was higher for women than for men (Ruitenberg et al., 2001 b).

Thus, also the role of gender in AD is still controversial as is the putative gender-related properties that may predispose or protect against the development of AD. Whether midlife risk factors have gender-specific effects on the development of AD is not known at the moment. Two earlier longitudinal studies on midlife BP or cholesterol only included males (Notkola et al., 1998; Launer et al., 2000).

2.8.2 Apolipoprotein E and gender

There is still a debate concerning whether possible gender differences exist in the effect of ApoE on the risk of AD. Some researchers have suggested an interaction between gender and ApoE, with women having a higher ApoE ε4-associated risk of AD than men (Farrer et al., 1997), but this has not been found by all researchers (Combarros et al., 1998). The view that that ApoE ε4 allele may lead to a greater risk for women than for men in the development of AD is largely based on a meta-analysis of 40 studies, which found a sex effect on risk of ApoE ε3/ε4 among Caucasians (Farrer et al., 1997). However, the meta-analysis also reported that women were more likely to develop AD than men across all ApoE genotypes, and the authors suggested that other factors such as estrogen, independently or in combination with certain ApoE genotypes, may account for some of the observed gender differences in the risk of AD.

2.8.3 Estrogen

An important gender-related difference that has been related to the risk of AD, is the dramatic decline of women's serum estrogen levels at menopause (Monk et al., 2000). The hypothesis that estrogen deficiency associated with menopause may contribute to the development of AD has gained support from studies reporting lower serum estrogen values in women with AD than in age-matched controls (Honjo et al., 1989). Estrogen is also known to possess neuroprotective and neurotrophic properties; it may enhance neuronal survival, inhibit apoptosis, promote synaptogenesis and synaptic plasticity, and improve cerebral blood flow (Garcia-Segura et al., 2001). Furthermore, estrogens may also influence the pathogenic processes of AD, mainly by affecting amyloid metabolism, by promoting the activity of the cholinergic system, and by reducing oxidative stress or cardiovascular risk (Skoog et al., 1999 a).

2.8.3.1 Estrogen and risk of AD

The epidemiological and biological rationale has sparked a number of studies on the effectiveness of ERT in preventing AD. Two recent meta-analyses and reviews on the effects of ERT on the risk of AD have reported a protective effect of ERT against AD (Yaffe et al., 1998; LeBlanc et al., 2001). In the latest meta-analysis (LeBlanc et al., 2001), including ten cross-sectional and two prospective studies, a 34 % decreased risk of AD (95 % CI 18–47 %) among estrogen users was reported. These studies are summarised in tables 6 and 7.

Even though the meta-analysis concluded that the risk of AD among postmenopausal women taking ERT was reduced, the ten case-control studies reviewed reported conflicting results. Some investigations showed a protective role of ERT (Henderson et al., 1994; Paganini-Hilland and Henderson, 1996; Harwood et al., 1999; Waring et al., 1999), others showed a non-significant protective effect for ERT (Broe et al., 1990; Mortel and Mayer, 1995), and others suggested no differences (Graves et al., 1990; Brenner et al., 1994), and finally, some demonstrated a non-significant but increased risk of AD (Heyman et al., 1984; Amaducci et al., 1986). It is important to note that the majority of the studies published so far are case-control studies with some important methodological limitations, including possible biases and lack of control for potential confounders, which limit interpretation of the results. For

example, several studies have not controlled for education. Women who use ERT are generally more educated (Matthews et al., 1996; Keating et al., 1999) and formal education has been reported to be protective against dementia. Several of the studies have only looked at the current use of ERT. Women with dementia might have been less likely to receive ERT because of concerns about compliance or potential interactions with complex medication regimens.

The strongest evidence for an association between ERT and AD comes from the prospective cohort studies, but only two that kind of studies appear to have been conducted so far (Tang et al., 1996; Kawas et al., 1997) (table 7). Both of the se studies observed protective effect for estrogen against development of AD.

Since the publication of the meta-analysis (LeBlanc et al., 2001), an additional study has been conducted; a population-based nested case control study that used computerised prescription records on the use of ERT. This study found no association between the ERT and a decreased risk of AD in a five-year follow-up (Seshadri et al., 2001) (table 7). While having many strengths, including objective methods to determine estrogen use, the study shares many of the limitations of the earlier case-control studies, for example lack of control for education. Another population-based study not included in the meta-analysis is the Italian Longitudinal Study of Aging (Baldereschi et al., 1998). The study cross-sectionally analysed the association between ERT and AD and reported an inverse relationship between ERT and AD, even after adjustments for several potential confounders (table 7).

Previous studies examining the relationship between ERT and cognitive decline and cognitive impairment have yielded inconsistent results (LeBlanc et al., 2001). Cognitive impairment has been defined by performance in a variety of neuropsychological tests, and not by any clinical or diagnostic concepts. Thus, the discrepancy between the results may at least partly be due to differences in cognitive instruments used, especially given that the effect of ERT on cognitive functions may be limited in selected cognitive domains. Currently, no studies appear to be published on the relation between ERT and MCI.

2.8.3.2 Estrogen as treatment for AD

Several small interventional trials have evaluated estrogen as a possible treatment for women diagnosed with AD. Findings in these studies have been reported as wholly or partly positive (e.g. Fillit et al., 1986; Honjo et al., 1989; Ohkura et al., 1994). An important limitation in these trials was the absence of a randomised and/or controlled design. Furthermore, all of these studies were constrained by very small sample sizes (seven to fifteen women) and had short duration (mean of six weeks).

The placebo-controlled, double-blind, randomised trials of estrogen in AD are summarised in table 8. Four small, randomised, double-blind placebo-controlled trials of estrogen have been reported (Honjo et al., 1993; Fillit et al., 1994; Birge et al., 1997; Asthana et al., 1999). Three of these studies reported improvement in estrogen group over placebo group in some outcome measures (Honjo et al., 1993; Fillit et al., 1994; Asthana et al., 1999). These studies are limited by small sample sizes, short duration of therapy, and non-specific and variable cognitive instruments.

Three somewhat larger randomised placebo-controlled trials of ERT treatment in women with manifest AD have been published so far (Henderson et al., 2000; Mulnard et al., 2000; Wang et al., 2000). Besides larger sample sizes, one advantage of these studies was the use of well-described multiple outcome measures. The follow-up times were, however, quite short, varying from three to twelve months. The conclusions drawn from these studies were that short-term ERT does not appear to slow the disease progression or improve cognitive functions in AD. However, the findings from these studies do not address possible long-term effects of estrogen on AD, possible interactions between estrogen and other treatment modalities, and putative effects of estrogen in preventing or delaying the onset of this disorder.

Table 6. The association of estrogen use and Alzheimer's disease in case-control studies.

Authors	Cases	Controls	Covariates	Adjusted OR (95% CI)
Heyman et al., 1984	4 definite and 36 probable AD, 15 % taking ERT	2 community controls per case, 8 % taking ERT	Age, education, and place of residence	2.4 (0.7 - 7.8) for current use compared with no current use
Amaducci et al., 1986	116 probable AD, 12 % taking ERT	97 community controls, 8 % taking ERT	Age and region of residence	1.7 (0.4 - 5.9) for use of estrogens in menopause compared with never use
Broe et al., 1990	83 probable and 87 possible AD, 8 % taking hormones	170 community controls, 11 % taking hormones	Age and region of residence	0.7 (0.4 - 5.9) for ≥ 6 months of hormonal treatment compared with no use
Graves et al., 1990	130 probable AD, 18 % taking ERT	139 friends and relatives of cases, 16 % taking ERT	Age	1.2 (0.5 - 2.6) for estrogen use at the time of dementia symptom onset compared with no use
Henderson et al., 1994	73 probable and 70 definite AD, 7% taking ERT	92 community controls, 18 % taking ERT	Age and education	0.3 (0.1 - 0.8) for current use of any estrogens compared with no current use. Majority used oral CEE
Mortel and Meyer, 1995	93 probable AD and 65 probable VaD, 11 % taking ERT	148 friends and relatives of cases and caregivers, 20 % taking ERT	Age	Current use compared with no current use, 0.6 (0.3 - 1.2) for AD, 0.5 (0.2 - 1.2) for VaD. Formulation not stated.
Paganini-Hilland Henderson, 1996	248 cases, dementia diagnoses on death certificates, 38 % taking ERT	5 controls who had died per case, 47 % taking ERT	Age, age at menarche and menopause, type of menopause, antihypertensive medication, stroke	0.65 (0.49 - 0.88) for ever use of any form compared with never use. Higher dose and longer duration; risk reduced more
Harwood et al., 1999	362 cases, 12 % taking ERT	192 controls, 41 % taking ERT	Age and education	Ever use compared with never use, Caucasians: 0.6 (0.3 - 1.0), Hispanic: 0.4 (0.2 - 1.0). Formulation not stated

Abbreviations: CEE = conjugated equine estrogen, ERT = estrogen replacement therapy.

Table 7. The association of estrogen use and Alzheimer's disease in population-based studies

Authors	Study population	Study design	Estrogen	Covariates	Adjusted RR/OR (95% CI)
Brenner et al., 1994	Population-based study at Group Health Cooperative of Puget Sound, Seattle, Washington; 107 probable or definite AD, 49 % taking ERT, mean age 78.7 years; 120 community controls, 48 % taking ERT, mean age 76.6 years	Case-control study	Ever use of any form; majority used CEE, Estrogen use based on recorded prescription data	Age and hysterectomy status	RR 1.1(0.6 - 1.8)
Tang et al., 1996	Manhattan Study of Aging; 167 probable AD in a cohort of 1124 community-dwelling women; 13 % taking ERT, mean age 74.2 years	Prospective study; mean follow-up time 1-5 y	Ever use of any form; majority used CEE	Age, education, ethnic group	RR 0.50 (0.25 - 0.90), RR 0.13 (0.02 - 0.92) if usage >1year
Kawas et al., 1997	Baltimore Longitudinal Study of Aging, 34 probable AD in a cohort of 472 community dwelling women, 45 % taking ERT, mean age 61.5 years	Prospective study; follow-up time up to 16 years	Ever use of any form; 212 used oral, 12 transdermal	Age, education	RR 0.46 (0.21 - 1.0)
Baldereschi et al., 1998	Italian Longitudinal Study of Aging, 92 patients with AD in a cohort of 1582 women, 11.6 % taking ERT, age 65 - 84 years	Cross-sectional study	Ever use of any form vs. never use	Age, education, age at menarche, age at menopause, smoking, alcohol habits at the age of 50 years, number of children	OR 0.28 (0.08 - 0.98)
Waring et al., 1999	Rochester Epidemiology Project, 222 patients with AD, 5 % taking ERT; 222 controls, 10 % taking ERT, mean age not stated	Case-control study	Use of any form of estrogen for more than 6 months; majority used CEE	Education, age at menopause	OR 0.42 (0.18 - 0.96)
Seschadri et al., 2001	United Kingdom-based General Practice Research database, 59 newly diagnosed probable or possible AD; 25 % taking ERT, mean age 66.7 years; 168 controls; 24 % taking ERT, mean age 65.2 years	Population-based nested case-control study; mean follow-up time 5.3 years	Current use of any form for more than 1 year; 36 used oral estrogens with progestins and 20 without, 12 transdermal estrogens, Computerised prescription records on ERT was used	Smoking, body mass index	OR 1.18 (0.59 - 2.37), OR 1.05 (0.32 - 3.44) if usage >5 years

Abbreviations: CEE = conjugated equine estrogen, CI = confidence interval, ERT = estrogen replacement therapy, OR = odds ratio, RR = risk ratio.

Table 8. Placebo-controlled, double-blind, randomised trials of estrogen in Alzheimer's disease.

Authors	Treatment duration	Estrogen	Outcome measures	Results
Honjo et al., 1993	14 women with AD 3 weeks	1.25 mg CEE/d orally	New Screening Test for Dementia. Hasagawa Dementia Scale, MMSE	Significant improvement on Hasagawa Scale but not on two other test in estrogen group compared with placebo group
Fillit et al., 1994,	8 women with AD 12 weeks	0.05 mg patch twice a week for three 4-week cycles, no drug given during the last week of each cycle	Specific outcome measures not stated	No significant difference between the groups
Birge et al., 1997	20 women with AD 9 months	0.625 mg CEE/d cycled with progesterone orally	Clinical impression of overall change	Significant improvement in estrogen group compared with placebo group
Asthana et al., 1999,	12 women with AD 8 weeks	Transdermal estradiol 0.05 mg/d	Buschke, Wechsler, Stroop, Verbal tests	Significant improvement on cued delayed recall and selective reminding test in estrogen group
Mulnard et al., 2000	97 hysterectomized women with AD 12 months	0.625 mg (n = 42) or 1.25 mg (n = 39) CEE /d orally	CGIG CDR MMSE	No significant difference in other tests, but CDR worsened among women taking estrogen
Henderson et al., 2000	36 women with AD 4-16 weeks	1.25 mg CEE/d, women with uterus received also 10 mg MPA/d for 14 d	ADAS-cog, CGIC, ADL/IADL	No significant difference between the treatment groups
Wang et al., 2000	47 women with AD 12 weeks	1.25 mg CEE/d orally	CASI, CIBIC-plus, CDR, BEHAVE-AD, HARS, HDRS, Cerebral blood flow	No significant difference between the groups
Asthana et al., 2001	20 women with AD 8 weeks	Estradiol 0.10 mg/d, transdermally	Stroop, Trail Making, Treisman, Buschke, Story Recall, Figure Copy/Memory, Visual Paired-Associates, Oculomotor Delayed Response, Boston Naming Test	Improvement on verbal and visual memory and attention in estrogen group

Abbreviations: ADAS-cog = Alzheimer's Disease Assessment Scale cognitive subscale; ADL = Activities of Daily Living; BEHAVE-AD = Behavioral Pathology in Alzheimer's Disease; CASI = Cognitive Ability Screening Instrument; CDR = Clinical Dementia Rating Scale; CEE = conjugated equine estrogen, CGIC = Clinical Global Impression of Change; CIBIC-plus = Clinical Interview-Based Impression of Change; HARS = Hamilton Anxiety Rating Scale; HDRS = Hamilton Depression Rating Scale; IADL = Instrumental Activities of Daily Living, MMSE = Mini-Mental State Examination, MPA = medroxyprogesterone acetate.

3 AIMS OF THE STUDY

As the proportion of elderly people in the population increases, AD will become an enormous public health problem. Interventions that could delay the onset of the disease, even modestly, would therefore have a major impact on public health. So far, few modifiable risk factors for AD have been identified. Thus, longitudinal, population-based studies are needed to determine the epidemiology and modifiable risk factors of AD. The aim of this study was to evaluate the impact of midlife vascular risk factors, the susceptibility gene ApoE, and gender-related factors on the development of AD in a prospective, population-based study setting. The study also attempted to evaluate risk factors for MCI, which is considered to represent a high-risk condition for AD.

The specific aims of the present study were:

1) To investigate the putative impact of midlife elevated blood pressure and serum cholesterol levels on the subsequent development of AD later in life. (Study I)

2) To evaluate the relation of midlife elevated blood pressure and serum cholesterol levels on the development of MCI. (Study II)

3) To study the relative importance and putative interrelationships between ApoE ε4 allele and midlife cholesterol and blood pressure as risk factors for AD. (Study III)

4) To evaluate the possible gender differences in the risk of AD related to ApoE ε4 allele and midlife vascular risk factors and vascular diseases later in life. (Study IV)

5) To study the relation of ERT with MCI and AD. (Study V)

4 PARTICIPANTS AND METHODS

4.1 Study population

Participants were derived from four separate and independent population-based random samples studied within the framework of the North Karelia Project and the FINMONICA (Finnish part of the Monitoring Trends and Determinants in Cardiovascular Disease) study in 1972, 1977, 1982, and 1987. These surveys were carried out to assess the levels of cardiovascular disease risk factors in two eastern provinces of Finland; North Karelia and Kuopio provinces. The formation of the original study samples has been previously described in detail (Vartiainen et al., 1991). In brief, for each survey an independent random sample was drawn from the national population register. In the 1972 and 1977 surveys, a random sample of 6.6 % of the population born during 1913–1947 (aged 25–59 years in 1972) was drawn in North Karelia and Kuopio. In 1982 and 1987 the sample involved people aged 25–64 years. The samples were stratified according to the WHO MONICA protocol so that at least 250 subjects of each sex and ten-year age group were chosen (WHO MONICA Project Principal Investigators, 1988). The common age range in the surveys was 30–59 years. The participation rates in these initial surveys were high, ranging from 77 % to 96 %.

Those individuals still alive, aged 65 to 79 at the end of 1997, and living in two geographically defined areas in or close to the towns of Kuopio and Joensuu (n = 2293), were the target of this study. From these subjects, a random sample of 2000 persons was invited to the re-examination carried out during 1998. The study was approved by the local ethics committee and written informed consent was obtained from all participants.

Altogether, 1449 (72.5 %) of the 2000 eligible individuals participated in the study. Individuals were asked to participate in the re-examination by letter. Among those who did not respond to two letters, a phone call was made to determine the reason for non-participation. Of those who did not participate in the screening phase (n = 551), 434 refused to participate for unknown reasons, 101 refused due to poor health, seven were in a long-term ward care, three had died, and six could not be contacted.

4.2 Methods

4.2.1 Midlife examination

The survey methods used during the midlife assessments were carefully standardised and complied with international recommendations. They also followed the World Health Organization (WHO) MONICA protocol in 1982 and 1987, and were comparable with methods used in 1972 and 1977 (WHO MONICA Project Principal Investigators, 1988). The survey included a self-administered questionnaire on socioeconomic factors, health behaviours including smoking and alcohol consumption, and medical history, including cardiovascular and cerebrovascular events and conditions diagnosed by a physician. Similarly, the use of antihypertensive medication was inquired about. The questionnaires were mailed to the participants before the visit and were returned to the survey site.

At the survey site, nurses specially trained for the survey checked the questionnaires to ensure that they were fully completed, and asked some additional questions, and measured height, weight, and BP. BMI was calculated as weight (in kilograms) divided by height (in meters) squared. Systolic and diastolic BP were measured from the right arm of the participants after they had been seated for five minutes. The fifth phase of the Korotkoff sounds was recorded as the diastolic BP. BP values were available for all 2000 individuals. The survey methods included single BP measurements for most of the participants. In the 1972 survey, only a single BP measurement was taken. For 186 (of 531) participants in the 1977 survey, and for all participants in the subsequent surveys, BP was measured twice. In summary, BP was measured twice in 571 individuals. After the measurements, a non-fasting venous blood specimen was taken to determine serum cholesterol and other parameters. Serum total cholesterol was determined from frozen serum samples using the Liebermann–Burchard method in 1972 and 1977, and from fresh serum samples using an enzymatic cholesterol oxidase/p-aminophenazone (CHOD-PAP) method (Monotest, Boehringer, Mannheim, Germany) in 1982 and 1987. The enzymatic assay method gave 2.4 % lower values than the Liebermann–Burchard method. Cholesterol values from the 1972 and 1977 surveys were thus corrected accordingly. All cholesterol determinations were made in the same central laboratory, and the laboratory data were standardised against national and

international reference laboratory values. Cholesterol values were available for all the 2000 individuals.

4.2.2 Re-examination in 1998

During the re-examination, dementia and cognitive impairment were diagnosed in a three-phase study design: a screening phase (Phase 1), a clinical phase (Phase 2), and a differential diagnostic phase (Phase 3). The screening phase was conducted during the first half of 1998 at the Department of Public Health and General Practice in the University of Kuopio, and at the North Karelia Project Office in Joensuu. The clinical and differential diagnostic phases were carried out at the Memory Research Clinic at the Department of Neurology, University of Kuopio and at the North Karelia Central Hospital in Joensuu between June 1998 and April 1999.

Phase 1 (screening phase). During the re-examination, the survey methods followed the WHO MONICA protocol, to be comparable with the methods used at the midlife assessments. The survey included a self-administered questionnaire as at the midlife examination. In addition to the assessments employed at midlife, use of lipid-lowering medication was inquired about. At the time of the midlife assessment, cholesterol-lowering drug treatment was not available, or its use was exceptional and rare, and it was not inquired about in the midlife questionnaires. Similarly, the use of ERT (starting age and duration of use) was inquired about only during the late-life assessment. Furthermore, the participants were studied for their ApoE genotypes. This was determined from blood leukocytes, from which DNA was extracted by a standard phenol-chloroform extraction, and ApoE genotypes were analysed by polymerase chain reaction, and *HhaI* digestion as described previously (Tsukamoto et al., 1993). Cognitive functions of the participants were screened using the Mini-Mental State Examination (MMSE) (Folstein et al., 1975).

Phase 2 (clinical phase). Subjects scoring ≤ 24 on the MMSE ($n = 280$) were invited to participate in the clinical phase. Ultimately, 240 of these subjects (86 %) took part in the clinical phase, which consisted of a medical history, thorough neurological and cardiovascular examinations performed by the physician (M.K.), and a detailed neuropsychological evaluation conducted by the neuropsychologist (E.-L.H.), which

included the Buschke Selective Reminding Test (Buschke and Fuld, 1974), the Logical Memory Test from the Wechsler Memory Scale–Revised (Wechsler 1987), the Boston Naming Test (Kaplan et al., 1983), the Vocabulary subtest of the Wechsler Adult Intelligence Scale (Wechsler 1981), the Verbal Fluency Test (Borkowski et al., 1967), the Copy a Cube Test (Goodglass and Kaplan, 1972), the Clock Setting Test (Goodglass and Kaplan, 1972), the Block Design subtest of the Wechsler Adult Intelligence Scale (Wechsler 1981), the Wisconsin Card Sorting Test using Nelson's version (Nelson 1976), and the Trail Making Test (Reitan 1958). The severity of cognitive decline was graded by the study physician according to the Clinical Dementia Rating scale (Berg 1998). A review board consisting of the study physician, the study neuropsychologist, and a senior neurologist (M.H.) ascertained the preliminary diagnosis based on all available information.

Phase 3 (differential diagnostic phase). Subjects with possible dementia were invited to the differential diagnostic phase, which included brain magnetic resonance imaging (MRI), blood tests, chest radiograph, electrocardiogram, and cerebrospinal fluid analysis. All data accumulated from the screening and clinical phases were carefully re-analysed by the review board before the final diagnosis was established.

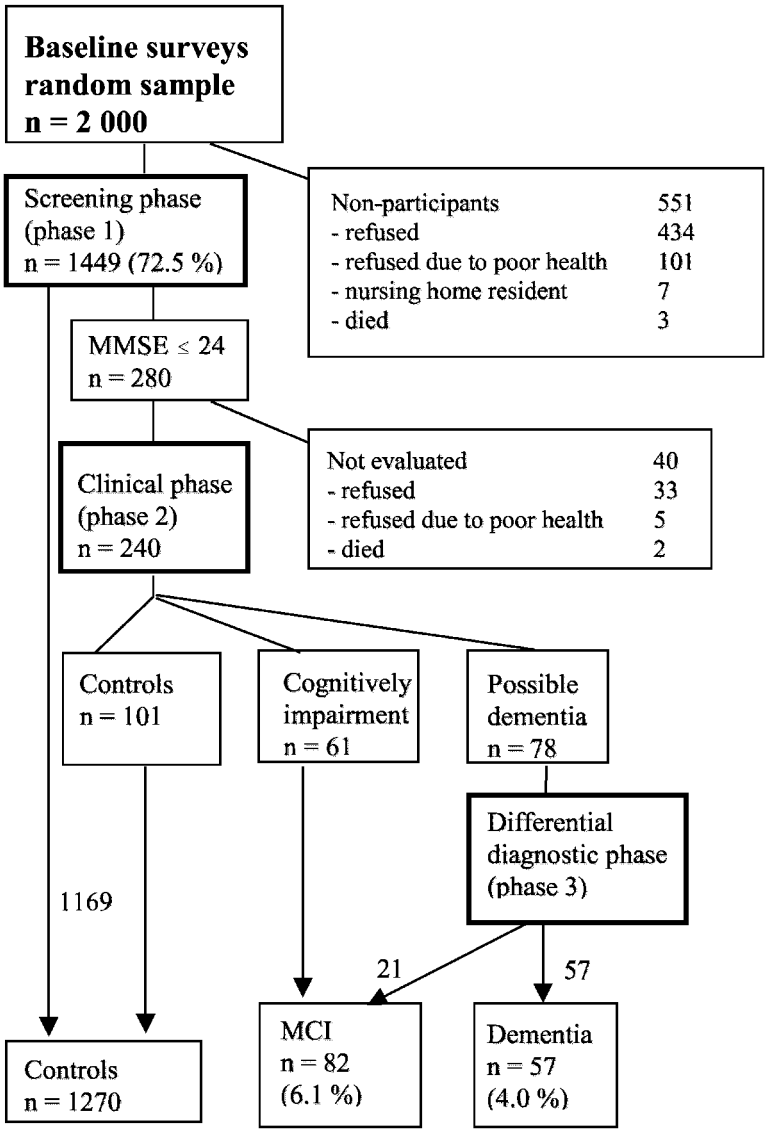

Figure 1. Formation of the study population. In the screening phase, participants scoring ≤ 24 on the Mini-Mental State Examination (MMSE) were invited to the clinical phase consisting of a medical history, neurological and cardiovascular examination, and detailed neuropsychological evaluation. Participants with possible dementia were invited to the differential diagnostic phase, which included magnetic resonance imaging, blood tests, chest radiograph, electrocardiogram, and cerebrospinal fluid analysis. MCI = mild cognitive impairment.

4.2.3 Diagnostic criteria

The diagnosis of dementia was based on the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th edn. (DSM-IV) (American Psychiatric Association, 1994) and the diagnosis of AD on the NINCDS-ADRDA criteria (McKhann et al., 1984). The diagnosis of VaD was based on the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINDS-AIREN) criteria (Román et al., 1993). For other types of dementia, the following criteria or guidelines were used: consensus diagnostic criteria for frontotemporal dementia (FTD) (Neary et al., 1998); consortium for dementia with Lewy bodies (DLB) (McKeith et al., 1996), and consensus criteria for alcohol dementia (Oslin et al., 1998).

The diagnostic criteria proposed by the MCADRC were used for diagnosing MCI (Petersen et al., 1995; Smith et al., 1996). These include 1) memory complaint by patient, family, or physician; 2) normal activities of daily living; 3) normal global cognitive function; 4) objective impairment of memory or one other area of cognitive function as evidenced by scores > 1.5 SD below the age-appropriate mean; 5) CDR score of 0.5; and 6) absence of dementia. As suggested by the MCADRC criteria, the cut-off point 1.5 SD below the norm in the neuropsychological tests was used as a guideline in the clinical assessment of cognitive performance (Petersen et al., 1995). The participants who had neither dementia nor MCI formed the control group.

4.2.4 Statistical analyses and formation of study groups (Studies I-V)

Midlife cholesterol and BP values were primarily used as categorical variables in the logistic regression models. Midlife serum cholesterol values (mmol/l) were classified into high (≥ 6.5) and normal (< 6.5). Midlife blood pressure values (mmHg) were classified into three categories: for systolic, normal (< 140), borderline (140–159), and high (≥ 160); and for diastolic, normal (< 90), borderline (90–94), and high (≥ 95).

Information about the sociodemographic characteristics, medication, vascular diseases, smoking, and alcohol consumption were obtained from the self-administered questionnaires, which were sent to the participants' homes before the assessments, and verified during the

examination. The variables were used in the analyses as follows: age (in years); formal education (in years); history of vascular diseases diagnosed by a physician (MI, cerebrovascular symptoms, and diabetes mellitus as separate variables) (yes/no); the use of antihypertensive and lipid-lowering medication (as separate variables) (yes/no); smoking (current smoker, former smoker, non-smoker); and alcohol consumption (not using alcohol, and using alcohol < 10 g/day, 10–30 g/day, > 30 g/day). In Study I, smoking and alcohol consumption were used as dichotomous variables (yes/no).

The analyses were conducted with the SPSS for Windows, v. 9.0 or 10.1 (SPSS Inc., Chicago, IL) software. Student's t tests and χ^2 tests of independence were used for assessing the mean and proportional differences. Multiple logistic regression analyses were used to calculate odds ratios (OR) with 95 % confidence intervals (CI). One-way analysis of variance (ANOVA) with Tukey *post hoc* analysis was used to compare the means between the three diagnostic groups (AD, MCI, controls). Repeated measures analyses of variance were used to analyse changes in the variables during the follow-up. Furthermore, in Study V the results were verified using logistic regression analysis of the LogXact program for Windows, v. 4.0 (Cytel Software Corporation), which is a special program for small sample sizes. The level of significance was $p < 0.05$ in all analyses.

Study I

First, after the dementia diagnoses were established, we identified individuals with AD and excluded the non-AD dementias from the analyses, because the focus of the study was AD, and the number of individuals with non-Alzheimer's dementia was small (n = 9). Differences between the individuals with AD and controls were analysed with Student's t tests and χ^2 tests as appropriate. The association between elevated midlife BP and cholesterol with subsequent AD was investigated with multiple logistic regression analysis using normal blood pressure and cholesterol as the reference category. The analysis was replicated adjusting for the confounding effects of age, BMI, education, history of MI and cerebrovascular symptoms (were used as indicators of vascular events), smoking, and alcohol consumption.

Study II

Second, after the MCI diagnoses were established, we wanted to analyse the differences between subjects with MCI and control subjects. Demented subjects were excluded from these analyses, and Student's t tests and χ^2 tests were used as appropriate. The association between elevated midlife cholesterol and BP and the development of MCI was investigated (as in Study I). To investigate the distribution of midlife vascular risk factors among demented individuals, those with MCI, and the control subjects, the demented subjects were included in the analyses, and a χ^2 test was used. The Mantel–Haenszel χ^2 test was used as a measure of linear association between these factors.

Study III

Third, the study participants were studied for their ApoE genotypes, and the analyses in Study III were restricted to persons from whom blood was available for ApoE genotyping, i.e. in all 48 AD patients and in 1239 (97 %) of the control subjects. Participants with MCI were excluded from these analyses because this group is heterogeneous, including individuals who may not become demented and also individuals who are likely to have incipient dementia. ApoE allele frequencies between the controls and AD patients were compared using χ^2 tests. The association between the ApoE ε4 allele and AD was studied using logistic regression analyses. To study whether ApoE ε4 allele was associated with AD independent of midlife hypercholesterolemia and elevated systolic BP, ApoE genotype (ε4 carriers vs. non-carriers) as well as cholesterol and systolic BP categories were included in the model. The adjusted model also included adjustments for age, history of MI and cerebrovascular symptoms, education, gender, smoking, and alcohol consumption. To investigate whether midlife vascular risk factors were associated with AD independently of the ApoE ε4 allele, midlife cholesterol, and BP categories (one at a time) and ApoE genotype (ε4 carriers vs. non-carriers) were entered in the model. The possibility of an interaction between ApoE and midlife cholesterol and BP were explored using logistic regression analyses. Furthermore, to investigate the joint effect of ApoE ε4 and high cholesterol and BP at midlife, ORs were calculated for this combination, using participants with no ApoE ε4 alleles and both normal cholesterol and BP as a reference group.

Study IV

The analyses in Study IV were restricted to persons from whom blood was available for the ApoE typing (as in Study III). To analyse the differences in sociodemographic and clinical characteristics between men and women, Student's t tests and χ^2 tests were used as appropriate. The associations of the ApoE ε4 allele, midlife BP and cholesterol, and vascular diseases later in life (MI, cerebrovascular symptoms, diabetes mellitus) with AD in men and women were studied (one at a time) using logistic regression analyses. The possibility of an interaction between gender and ApoE and vascular risk factors were explored using logistic regression analyses.

Study V

Altogether, 875 of the 1449 participants were women, and the analyses in Study V were restricted to them. The use of ERT was inquired about (starting age and duration of use) by the self-administered questionnaire. For 75 women there was no information available about ERT, and the analyses were restricted to those women for whom the data were available (n = 800).

Subjects were classified into two groups according to whether or not they had received ERT. Differences between these groups were analysed using Student's t tests and χ^2 tests as appropriate. The associations between ERT usage with MCI and AD were studied using logistic regression analyses. Subjects with MCI as well as patients with AD were compared to the control subjects. Because age and education may influence the association between ERT and cognitive impairment, a second model was performed, making adjustments for age and education. The effects of cholesterol and systolic BP levels were accounted for in the following model, and finally, the analyses were carried out controlling also for history of MI and cerebrovascular symptoms, and ApoE ε4 allele status (carriers vs. non-carriers).

5 RESULTS

5.1. General characteristics of the study population

Most of the study subjects participating in the re-examination during 1998 were given their midlife examination during 1972 or 1977, and the mean follow-up time for the study population was 21 years. The mean age at midlife assessment for the study participants was 50 years (range 40–64) and 71 years (range 65–80) at re-examination. The proportion of women (62 %) was equal to that in the general population in this age group in the study area (61–64 %) (Koivisto et al., 1992).

Comparison of participants with non-participants in the late-life visit showed that participants were younger, more educated, and had lower BMI (table 9). At midlife, non-participants had higher systolic BP, diastolic BP, and serum total cholesterol compared to those participating in the late-life examination. They did not differ significantly with regard to gender, smoking, alcohol consumption, use of antihypertensive medication, or prevalence of MI, cerebrovascular symptoms, or diabetes mellitus at midlife (results not shown).

5.2 Prevalence of Alzheimer's disease and mild cognitive impairment

A total of 57 subjects (4 %) were diagnosed as having dementia, out of whom 48 fulfilled the diagnosis of probable or possible AD. All the AD patients displayed generalised and/or medial temporal atrophy, and none had significant vascular pathology on magnetic resonance scans. Isolated, minor lacunae or periventricular white matter signal changes were not considered as exclusion criteria for AD. All scored four or less on the Hachinski Ischemic Score. Four patients were diagnosed as having vascular dementia, two with Parkinson's disease with dementia, two with alcohol dementia, and one with frontotemporal dementia.

Altogether, 82 subjects (6.1 %) were diagnosed as having MCI. If memory tests were used as the only neuropsychological criteria for the diagnosis of MCI, six subjects would have been excluded, leaving 76 (5.6 %) in the MCI group. The characteristics of these 76 subjects did not differ from the original MCI group. The six subjects who did not score below the 1.5 SD cut-off on the memory tests had, however, somewhat milder memory impairment.

Table 9. Sociodemographic and clinical characteristics of the participants vs. non-participants.

Characteristic	**Participants** (n = 1409)	**Non-participants** (n = 591)	**p value**
Male / female*	37.9 / 62.1	36.5 / 63.5	0.57
Follow-up time (years)	20.9 (4.9)		
Age (years) - midlife - late-life	 50.4 (6.0) 71.3 (4.0)	 51.2 (5.9)	 **0.007**
Education (years)	8.4 (3.5)	5.4 (1.8)	**<0.001**
Mini-Mental State Examination	25.9 (2.4)		
Body mass index (kg/m^2) - midlife - late-life	 26.6 (3.7) 27.8 (4.3)	 27.2 (4.5)	 **0.002**
Systolic blood pressure (mmHg) - midlife - late-life	 144.3 (19.9) 151.4 (23.3)	 150.2 (21.7)	 **< 0.001**
Diastolic blood pressure (mmHg) - midlife - late-life	 89.2 (10.9) 80.4 (11.2)	 91.7 (11.3) 7.0 (1.3)	 **< 0.001**
Cholesterol (mmol/l) - midlife - late-life	 6.7 (1.2) 5.8 (1.0)	 7.0 (1.3)	 **< 0.001**

Values are means (SD) and t test was used unless otherwise indicated.
*Percentages and χ^2 test were used. Significant results are marked with bold font.

5.3 Vascular risk factors and Alzheimer's disease (Study I)

5.3.1 Sociodemographic and clinical characteristics

Patients with AD were significantly older, and they also had a lower degree of formal education than controls (table 10). Patients with AD had higher BMI, systolic BP, and serum total cholesterol at midlife than controls, but at re-examination there were no significant differences in these values between the two groups. There was no difference in diastolic BP between patients with AD and controls at midlife or at re-examination.

Table 10. Sociodemographic and clinical characteristics of the patients with Alzheimer's disease vs. controls.

Characteristic	**Alzheimer's disease** (n = 48)	**Controls** (n = 1352)	**p value**
Age (years)			
- midlife	54.0 (4.7)	50.2 (6.0)	**< 0.001**
- late-life	74.7 (3.8)	71.1 (4.0)	**< 0.001**
Education (years)	6.7 (2.7)	8.5 (3.5)	**< 0.001**
Body mass index (kg/m^2)			
- midlife	27.6 (4.0)	26.5 (3.7)	**0.04**
- late-life	27.8 (4.4)	27.8 (4.3)	0.99
Systolic blood pressure (mmHg)			
- midlife	152.7 (18.2)	143.9 (19.8)	**0.002**
- late-life	150.4 (23.5)	151.5 (23.4)	0.75
Diastolic blood pressure (mmHg)			
- midlife	91.2 (9.5)	89.1 (10.9)	0.18
- late-life	80.4 (11.5)	80.5 (11.2)	0.97
Cholesterol (mmol/l)			
- midlife	7.2 (1.0)	6.7 (1.2)	**0.001**
- late-life	6.0 (1.0)	5.8 (1.0)	0.23

Values are means (SD) and t test was used unless otherwise indicated.
*Percentages and χ^2 test were used. Significant results are marked with bold font.

5.3.2 Blood pressure and cholesterol and risk of AD

High systolic BP at midlife was a significant risk factor for AD in later life (table 11), and this remained true after all the adjustments (model 2). Borderline high systolic BP at midlife also increased the risk (model 1), but after adjustments this association was no longer significant. Midlife diastolic BP had no significant effect on the risk of AD in any of the models. High serum cholesterol level at midlife was a significant risk factor for AD later in life, even after all the adjustments were made.

Table 11. Association of midlife blood pressure and cholesterol levels with late-life Alzheimer's disease.

Characteristic	Odds ratio (95 % confidence interval)	
	Model 1	Model 2
Systolic blood pressure (mmHg)		
< 140 (normal)	1.0	1.0
140 - 159 (borderline)	**2.1 (1.0–4.3)**	2.1 (0.8–5.0)
≥ 160 (high)	**3.1 (1.4–6.6)**	**2.8 (1.1–7.2)**
Diastolic blood pressure (mmHg)		
< 90 (normal)	1.0	1.0
90 - 94 (borderline)	1.0 (0.47–2.3)	1.4 (0.6–3.5)
≥ 95 (high)	1.7 (0.88–3.2)	1.7 (0.8–3.6)
Cholesterol (mmol/l)		
< 6.5 (normal)	1.0	1.0
≥ 6.5 (high)	**2.9 (1.5–5.8)**	**2.2 (1.0–4.7)**

Multiple logistic regression analysis was used to create the models. Model 1 gives univariate odds ratios of Alzheimer's disease; model 2 includes adjustments for age, body mass index, education, history of myocardial infarction and cerebrovascular symptoms, smoking and alcohol consumption. Significant results are marked with bold font.

Because high systolic BP and cholesterol were significant risk factors for AD, we evaluated the added risk related to possessing both these risk factors. Participants with both risk factors (systolic BP ≥ 160 mmHg and cholesterol ≥ 6.5 mmol) at midlife had a significantly higher risk for AD than did those with either of these risk factors alone (systolic BP ≥ 160 mmHg or cholesterol ≥ 6.5 mmol). After all the adjustments (model 2), OR for this combined group vs. the single risk factor group was 3.5 (95% CI 1.6–7.9). The risk of AD in the combined risk factor group over the single risk factor group was significantly higher even when participants with borderline high systolic BP (140–159 mmHg) were included (cut-off for systolic BP 140 mmHg) (adjusted OR = 2.8, 95% CI 1.3–5.9).

All the analyses were controlled for age at the time when risk factor data were collected, because the effects of these midlife variables were the focus of this study. The length of follow-up did not differ between participants who developed AD and those who did not; thus controlling for age at re-examination did not change the results (data not shown).

5.3.3 Medical history and AD

Patients with AD were more likely to have been treated with antihypertensive drugs at midlife than controls (27 % vs. 14 %, p = 0.02), but at the late-life re-examination there was no difference between the groups (40 % vs. 36 %, respectively, p = 0.62). At the re-examination, there was no difference in the usage of cholesterol-lowering drug treatments between patients with AD and controls. At the re-examination, patients with AD were significantly more likely to have a history of MI (38 % vs. 14 %, p < 0.001) and cerebrovascular symptoms (almost invariably expressed as transient ischemic attack) (19 % vs. 7 %, p = 0.004), and less likely to be alcohol users (46 % vs. 71 %, p = 0.003) than controls. There was no significant difference between patients with AD and controls in the prevalence of diabetes mellitus or smoking.

5.4 Vascular risk factors and mild cognitive impairment (Study II)

5.4.1 Sociodemographic and clinical characteristics

Participants with MCI were significantly older and had a lower degree of formal education than controls (table 12). At midlife, participants with MCI had significantly higher serum total cholesterol and a trend toward higher systolic BP than controls, but at re-examination later in life there was no difference in these values. Participants with MCI had a wider distribution (SD ± 23.2) of systolic BP values at midlife than control subjects (SD ± 19.6) (Levene's test for equality of variances: F = 5.4, p = 0.02). There was no difference in diastolic BP or BMI between participants with MCI and controls at midlife or re-examination.

Table 12. Sociodemographic and clinical characteristics of participants with mild cognitive impairment (MCI) and controls.

Characteristic	**Participants with MCI** (n = 82)	**Controls** (n = 1270)	**p value**
Age (years)			
- Midlife	51.7 (5.8)	50.1 (6.0)	**0.02**
- Late-life	72.8 (4.1)	71.0 (3.9)	**< 0.001**
Education (years)	6.8 (2.4)	8.8 (3.5)	**< 0.001**
Body mass index ($kg/m^{2)}$			
- Midlife	27.1 (3.6)	26.5 (3.7)	0.13
- Late-life	28.7 (6.2)	27.7 (4.1)	0.17
Systolic blood pressure (mmHg)			
- Midlife	148.4 (23.2)	143.6 (19.6)	0.07
- Late-life	151.5 (22.2)	151.5 (23.5)	0.98
Diastolic blood pressure (mmHg)			
- Midlife	90.2 (14.8)	89.6 (11.3)	0.83
- Late-life	80.0 (10.8)	80.5 (11.2)	0.72
Cholesterol (mmol/l)			
- Midlife	7.2 (1.2)	6.7 (1.2)	**< 0.001**
- Late-life	5.9 (1.2)	5.8 (1.0)	0.67

Values are means (SD) and t test was used unless otherwise indicated.
*Percentages and χ^2 test were used. Significant results are marked with bold font.

5.4.2 Blood pressure and cholesterol and risk of MCI

High serum cholesterol (≥ 6.5 mmol/l) at midlife was a significant risk factor for MCI later in life (table 13). There was a trend toward high systolic BP (≥ 160 mmHg) at midlife in subjects with MCI compared to control subjects (26.8 % vs. 18.9 %, p = 0.07). Borderline high systolic BP (140 to 159 mmHg) and elevated diastolic BP at midlife had no effect on the risk of MCI. Multiple adjustments (model 2) did not change the results.

A total of 61 % of the individuals with MCI had high cholesterol or high systolic BP at midlife. The OR of MCI for this single risk factor group was 1.9 (95% CI 1.1–3.3) after all the adjustments (as in model 2 in table 13). Individuals with combined risk factors (both systolic BP ≥ 160 mmHg and cholesterol level ≥ 6.5 mmol/l) at midlife had a significantly higher risk of MCI than the low-risk group (systolic BP < 160 mmHg and cholesterol < 6.5 mmol/l) (adjusted OR = 2.2, 95 % CI 1.2-4.8), but there was no difference in the risk between the combined risk factors group and the single risk factor group.

Table 13. Association of midlife blood pressure and cholesterol levels with late-life mild cognitive impairment.

Characteristic	**Odds ratio (95 % confidence interval)**	
	Model 1	**Model 2**
Cholesterol (mmol/l)		
< 6.5 (normal)	1.0	1.0
≥ 6.5 (high)	**2.0 (1.2–3.2)**	**1.8 (1.1–2.8)**
Systolic blood pressure (mmHg)		
< 140 (normal)	1.0	1.0
140–159 (borderline)	0.8 (0.5–1.4)	0.8 (0.4–1.3)
≥ 160 mmHg (high)	1.5 (0.8–2.5)	1.2 (0.7–2.2)
Diastolic blood pressure (mmHg)		
< 90 (normal)	1.0	1.0
90–94 (borderline)	0.9 (0.5–1.7)	1.1 (0.6–2.1)
≥ 95 (high)	1.2 (0.7–2.0)	1.3 (0.7–2.3)

Multiple logistic regression analyses were used to create the models. Model 1 gives univariate odds ratios of MCI; model 2 includes adjustments for age, body mass index, education, history of myocardial infarction and cerebrovascular symptoms, and smoking and alcohol consumption. Significant results are marked with bold font.

5.4.3 Medical history and MCI

The prevalence of MI and cerebrovascular symptoms tended to be higher in individuals with MCI than in control subjects, but the differences between the groups were not significant either at midlife (MI: 6.3 % vs. 3.8 %, $p = 0.24$; cerebrovascular symptoms: 3.8 % vs. 1.2 %, $p = 0.16$) or at late-life (MI: 20.3 % vs. 13.7 %, $p = 0.18$; cerebrovascular symptoms: 9.2 % vs. 6.5 %, $p = 0.35$). However, individuals with MCI had more often been given a diagnosis of hypertension at midlife than controls (43.2 % vs. 31.6 %, $p = 0.03$), although there was no difference in antihypertensive drug treatment between MCI and controls (12.2 % vs. 14.5 %, $p = 0.57$). At the late-life examination there was no difference between the groups either in the prevalence of hypertension or the use of antihypertensive or cholesterol-lowering drugs. There was no significant difference in smoking or history of diabetes mellitus at midlife or late-life. At midlife there was no difference in alcohol consumption between the groups, but at late-life examination the controls reported consuming more alcohol than individuals with MCI did (72.5 % vs. 52.5 %, $p < 0.001$).

5.4.4 MCI diagnosis with exclusion of health problems

In further analyses, we excluded those individuals who had health problems that could have had an impact on cognitive function. Information about these health problems was obtained during the clinical phase, which included taking a medical history and performing clinical examinations, and hospital records were also used to check the information. Ultimately, three individuals with a history of brain ischemia, two with cerebral haemorrhage, one with Parkinson's disease, four with depression, two with other psychiatric illnesses, three with chronic abuse of alcohol, and three with serious systemic illness were excluded, leaving a total of 64 (4.8 %) participants who met the criteria for diagnosis of MCI. The relationship between midlife cholesterol and BP and MCI after exclusion of these other health problems yielded virtually the same results (data not shown) as the overall analysis. However, the association between MCI and the combined risk factors (adjusted OR = 2.4, 95 % CI 1.1–5.0) and also the single risk factor (adjusted OR = 2.1, 95 % CI 1.1–5.5) were slightly stronger in this exclusionary analysis than in the overall analysis.

5.4.5 Vascular characteristics among persons with dementia, mild cognitive impairment, and controls

Figure 2 presents midlife cardiovascular characteristics of the demented, MCI, and control groups. The distribution of cholesterol, systolic BP, and risk factor categories differed significantly between the three groups: demented subjects most often had high cholesterol, high systolic BP, and combined risks at midlife, and the controls most seldom had these characteristics. At midlife, subjects with MCI had cholesterol levels and systolic BP that were higher than those who remained cognitively normal but lower than those who developed dementia. Thus, subjects with MCI had a risk factor profile that was intermediate between the demented persons and the control, with a significant linear trend over the three categories ($p < 0.001$ for linear trend). A positive history of MI and cerebrovascular symptoms was highest among demented subjects, second highest among individuals with MCI, and lowest among controls ($p < 0.001$ for linear trend).

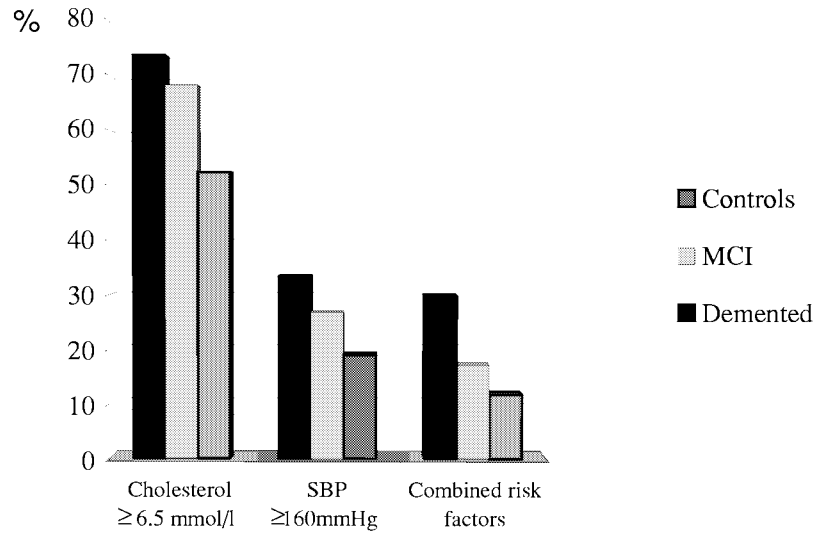

Figure 2. Midlife vascular characteristics among demented, individuals with MCI and controls. $P < 0.001$ for linear trend. Combined risk factors = systolic blood pressure (SBP) ≥ 160 mmHg and cholesterol ≥ 6.5 mmol/l.

5.5 Blood pressure and cholesterol changes during follow-up

We analysed changes in BP and cholesterol values during the average follow-up period of 21 years (from midlife assessment to the re-examination) in the three diagnostic groups (AD, MCI, and controls) using repeated measures ANOVA. Longitudinal measurements of BP and cholesterol values are presented in figure 3. In general, there was a significant change in systolic BP values during the follow-up ($F = 3.7$, $p < 0.05$). Furthermore, the systolic BP change was dependent on the diagnostic group ($F = 5.3$, $p = 0.005$). At midlife, there was a significant difference in the systolic BP values between the three groups ($F = 6.9$, $p = 0.001$); individuals who later developed AD had the highest values, and controls had the lowest. According to Tukey *post hoc* analysis, the difference between the AD group and controls was significant ($p = 0.005$), and the difference between the MCI group and controls approached significance ($p = 0.07$), whereas the AD group and the MCI group did not differ from each other with regard to midlife systolic BP ($p = 0.46$). During the follow-up, systolic BP values decreased in the AD group but increased in the other groups. At the late-life assessment, during the time of the diagnosis, patients with AD had lower values than the individuals with

MCI and controls, even though the differences were not statistically significant ($F = 0.05$, $p = 0.95$).

Diastolic BP decreased significantly during the follow-up ($F = 158.1$, $p < 0.001$), and the change was not dependent on the diagnostic group ($F = 1.61$, $p = 0.20$). There were no significant differences in diastolic BP values at midlife ($F = 1.67$, $p = 0.19$) or at late-life examination ($F = 0.06$, $p = 0.94$) between the groups.

During the follow-up period, the cholesterol values decreased significantly ($F = 229.1$, $p < 0.001$), and the change was dependent on the diagnostic group ($F = 7.14$, $p = 0.001$). At midlife, the cholesterol values differed between the three diagnostic groups ($F = 11.4$, $p < 0.001$), and both the AD group ($p = 0.005$) and MCI group ($p = 0.001$) differed significantly from controls, but not from each other ($p = 0.99$). At late-life, there were no differences in cholesterol values between the groups ($F = 0.81$, $p = 0.45$).

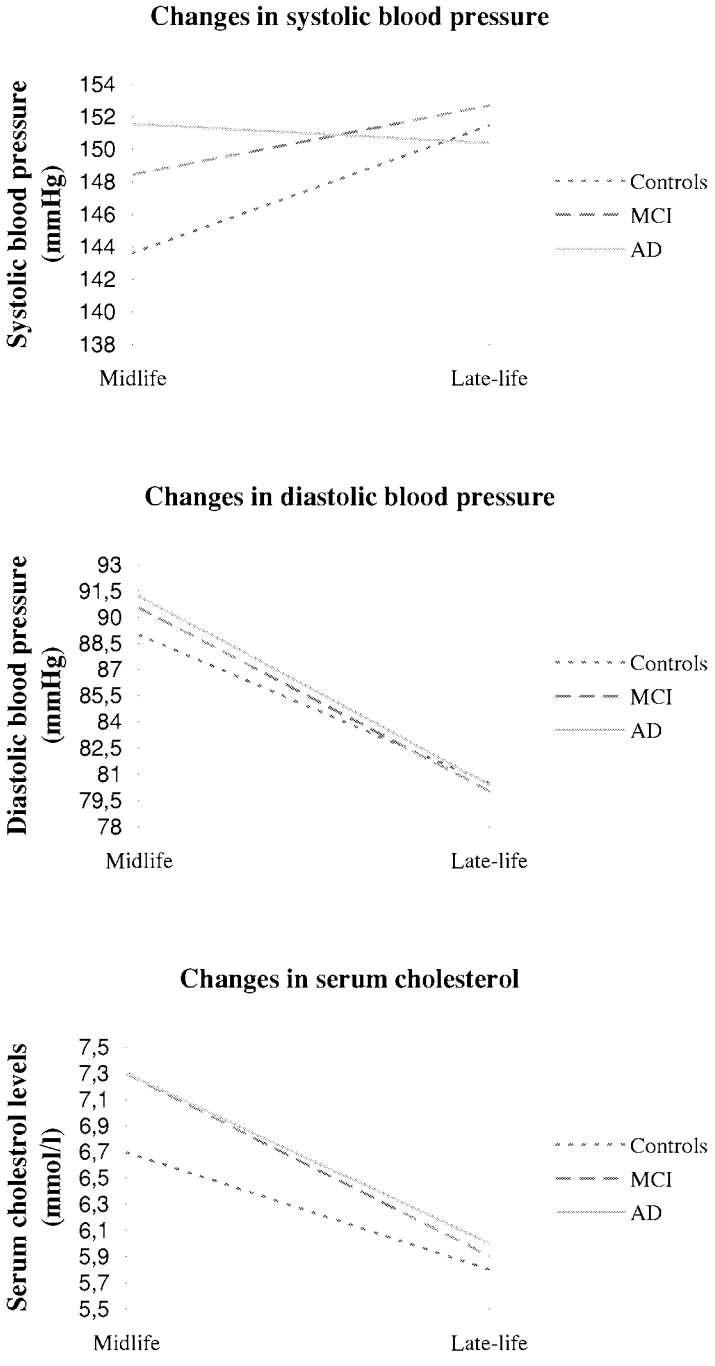

Figure 3. Changes in blood pressure and cholesterol values from the midlife assessment to the late-life examination for controls, subjects diagnosed to have mild cognitive impairment (MCI) and Alzheimer's disease (AD) at late-life.

5.6 ApoE and vascular risk factors and Alzheimer's disease (Study III)

5.6.1 The risk associated with the ApoE ε4 allele

The ApoE ε4 non-carriers tended to be older at the late-life assessment than the ε4 carriers (71.3 vs. 70.9 years, p = 0.05). The ApoE ε4 carriers had higher serum total cholesterol levels at midlife (6.85 vs. 6.68 mmol/l, p = 0.005) and late-life (5.90 vs. 5.78 mmol/l, p = 0.04) than non-carriers. There were no differences in systolic or diastolic BP, BMI, history of MI or cerebrovascular symptoms in midlife or late-life between the ApoE ε4 carriers and non-carriers.

The frequencies of the ApoE polymorphisms in the sample including patients with AD and controls were as follows: ε2/3, 6.3 %; ε3/3, 58.1 %; ε2/4, 1.4 %; ε3/4, 29.8 %; ε4/4, 4.0 %. Table 14 describes these frequencies separately for the patients with AD and controls. The allele frequencies were 3.9 % for ε2, 76.3 % for ε3, and 19.8 % for ε4.

Table 14. ApoE genotypes in the patients with Alzheimer's disease and controls.

ApoE genotype	**Alzheimer's disease** (n = 48)	**Controls** (n = 1239)
2/3	1 (2.1 %)	85 (6.9 %)
3/3	22 (45.8 %)	726 (58.6 %)
2/4	-	18 (1.5 %)
3/4	21 (43.8 %)	362 (29.2 %)
4/4	4 (8.3 %)	48 (3.9 %)

The frequency of the ApoE ε4 allele was higher in patients with AD than in controls. The frequencies of ApoE ε2/ε3/ε4 alleles were 4.2/76.6/19.2 % for the controls and 1.0/68.8/30.2 % for the AD patients, respectively (χ^2 = 8.7, p = 0.013). 52.1 % of the patients with AD were ApoE ε4 carriers (one or two ε4 alleles) compared to 34.5 % of the controls (χ^2 = 6.2, p = 0.013).

The ApoE ε4 allele was a significant risk factor for AD (crude OR = 2.1, 95 % CI 1.2–3.7). Adjusting for age, midlife cholesterol and systolic BP, history of MI or cerebrovascular symptoms, gender, education, smoking, and alcohol consumption did not change the association (adjusted OR = 2.1, 95 % CI 1.1–4.1).

The association between ApoE ε4 and AD also remained unchanged when adjusting for midlife diastolic BP, or when midlife cholesterol and BP were used as continuous variables instead of categorical variables in the main analysis. Similarly, adjusting for these risk factors measured at re-examination did not modify the association between ApoE ε4 and AD (data not shown).

5.6.2 Association between vascular risk factors and AD, adjusted for ApoE

Adjustment for the ApoE ε4 allele did not modify the risk of AD related to high midlife cholesterol (≥ 6.5 mmol/l) or systolic BP (≥ 160 mmHg). The crude OR for AD of high cholesterol was 2.9 (95 % CI 1.4–5.7), and after controlling for age, ApoE ε4 status (carriers vs. non-carriers), education, gender, smoking, and alcohol consumption, the OR was 2.8 (95 % CI 1.2–6.7). The crude OR for high systolic BP was 2.4 (95 % CI 1.1–5.3), and after all the adjustments the OR was 2.6 (95 % CI 1.1–6.6).

A history of late-life MI was a significant risk factor for AD (crude OR = 2.0, 95 % CI 1.3–3.1), even after making multiple adjustments as above (adjusted OR = 2.1, 95 % CI 1.1–4.5). Late-life cerebrovascular symptoms were also associated with AD (crude OR = 2.8, 95 % CI 1.3–6.5), but after adjustment for all the confounders, the association was not significant (OR = 2.4, 95 % CI 0.9-6.5). Neither a history of MI nor cerebrovascular symptoms at midlife were significant risk factors for AD (data not shown).

5.6.3 Combined effects of vascular risk factors and ApoE

The putative interactions between ApoE and midlife cholesterol and BP were tested using logistic regression analyses. No significant interactions were found between ApoE and cholesterol, ApoE and systolic BP, or the combination of all these risks for the development of AD. The combinations of these risk factors increased the risk in an additive manner, that

is, the risk increased as more risk factors were added. The crude OR for AD in participants with the combination of the ApoE ε4 allele, high midlife cholesterol, and elevated midlife systolic BP compared with participants having none of these factors was 11.0 (95 % CI 3.6–34.0); after adjustment for age, education, gender, smoking, and alcohol use, the OR was 8.4 (95 % CI 2.0–36.0).

5.7 Does gender make a difference? (Study IV)

5.7.1 Sociodemographic and clinical characteristics of men and women

The study population included 492 men and 795 women. The prevalence of AD and the frequency of the ApoE ε4 allele did not differ between men and women (table 15). Women were older, and had higher BMI, serum total cholesterol, and systolic BP at the late-life assessment than men. In contrast, men had higher diastolic BP and higher prevalence of MI both at midlife and at late-life than women. There was no difference in the prevalence of cerebrovascular symptoms or diabetes mellitus between the genders at midlife or late-life.

At midlife, women used antihypertensive drug treatment more often than men (16.4 % vs. 12.2 %, $p = 0.041$), but at late-life there was no difference in the use of antihypertensive drug treatment, which was very common in both men and women (35.4 % vs. 36.4 %, $p = 0.72$). At late-life, there was no difference between the men and women in the use of cholesterol-lowering drugs (14.4 % vs. 15.7 %, $p = 0.56$).

Table 15. Clinical characteristics of male and female participants.

Characteristic	**Males** (n = 502)	**Females** (n = 818)	**p value**
* Alzheimer's disease	3.8	3.5	0.82
*Apolipoprotein E ε4 allele	38.3	33.9	0.10
Age (years) - Midlife - Late-life	 50.0 70.9	 50.6 71.5	 0.12 **0.005**
Education (years)	8.9	8.5	**0.04**
Body mass index (kg/m$^{2)}$ - Midlife - Late-life	 26.5 (3.1) 27.1 (3.6)	 26.6 (4.0) 28.2 (4.6)	 0.61 **< 0.001**
Cholesterol (mmol/l) - Midlife - Late-life	 6.7 (1.1) 5.5 (0.9)	 6.8 (1.3) 6.0 (1.0)	 0.10 **< 0.001**
Systolic blood pressure (mmHg) - Midlife - Late-life	 143.6 (18.3) 148.9 (23.2)	 144.6 (20.7) 152.9 (23.3)	 0.36 **0.002**
Diastolic blood pressure (mmHg) - Midlife - Late-life	 90.4 (11.2) 81.6 (11.7)	 88.5 (10.7) 79.7 (10.8)	 **0.002** **0.001**
*Myocardial infarction - Midlife - Late-life	 5.1 21.8	 1.1 10.6	 **< 0.001** **< 0.001**
*Cerebrovascular symptoms - Midlife - Late-life	 1.4 8.1	 1.3 6.7	 0.85 0.35
*Diabetes mellitus - Midlife - Late-life	 1.7 8.1	 0.8 5.8	 **0.03** 0.36

Values are means (SD) and t test was used unless otherwise indicated.
*Percentages and χ^2 test were used. Significant results are marked with bold font.

5.7.2 The risk of AD associated with the ApoE ε4 allele

Of the men with AD, 63.2 % were ApoE ε4 carriers compared to 37.4 % of the non-demented men (p = 0.024). In women, 44.8 % of AD patients had the ApoE ε4 allele compared to 32.8 % of the non-demented women (p = 0.17).

The frequencies of the ApoE ε2/ε3/ε4 alleles were 0.04/ 0.76/0.21 in the non-demented men and 0/0.61/0.40 in the men with AD (p = 0.015). In the non-demented women, the frequencies of ApoE ε2/ε3/ε4 alleles were 0.05/0.78/0.18 and in the women with AD 0.02/0.74/0.24 (p = 0.35).

The ApoE ε4 allele was a significant risk factor for AD in men, even after adjusting for age, education, and vascular risk factors and diseases (midlife systolic BP and cholesterol, and late-life MI and cerebrovascular symptoms) (table 16, models 1–3). In women, the association between the ApoE ε4 allele and AD was less robust than in men; the ApoE ε4 allele was a significant risk factor for AD after adjusting for age and education (table 16, model 2), but in the univariate model (model 1), and after all the adjustments (model 3), it only approached significance.

Table 16. Association of ApoE ε4 allele and Alzheimer's disease in men and women.

	Odds ratio (95% confidence interval)		
	Model 1	**Model 2**	**Model 3**
Men (n = 492)	**2.9 (1.1–7.4)**	**3.5 (1.3–9.2)**	**3.9 (1.3–11.4)**
Women (n = 795)	1.7 (0.8–3.5)	**2.0 (1.0–4.4)**	1.9 (0.8–4.6)

Multiple logistic regression analysis was used to create the models. Model 1 gives crude odds ratios with 95 % confidence intervals for AD, Model 2 includes controlling for age and education, Model 3 for age, education, midlife cholesterol and systolic blood pressure, and history of myocardial infarction and cerebrovascular symptoms.

Further analyses on the effect of ApoE ε4 in people not having received antihypertensive drug treatment at midlife were carried out. This was done because it had been suggested that antihypertensive medication might modify the effects of the ApoE ε4 allele (Guo et al., 2001), and because women used antihypertensive drugs at midlife significantly more often than men in our study. Interestingly, the effect of ApoE ε4 allele was stronger in this sub-

group; in women (n = 665), the OR for AD was 2.6 (95 % CI 1.0–7.4) and in men (n = 472), the OR was 5.3 (95 % CI 1.5–18.4) after controlling for age, education, midlife systolic BP, and cholesterol, and late-life MI and cerebrovascular symptoms.

5.7.3 Midlife cholesterol and blood pressure and the risk of AD

Midlife high cholesterol was a significant risk factor for AD both in men and women, even after adjustments were made (table 17). High midlife systolic BP significantly increased the risk of late-life AD in men, even after making multiple adjustments. In women, the risk of AD related to high midlife systolic BP was in the same direction but did not reach a level of statistical significance. However, women who developed AD had more often received antihypertensive drug treatment at midlife than their counterparts without dementia (31 % vs. 16 %, p = 0.036) supporting the association between the history of hypertension and AD also in women. There was no difference in the use of antihypertensive drugs at midlife between men who developed AD and the non-demented men (21 % vs. 12 %, p = 0.213). Diastolic BP was not associated with the development of AD in either gender (table 17).

Table 17. Association between midlife cholesterol and systolic blood pressure and Alzheimer's disease later in life in men and women.

Characteristic	Odds ratio (95 % CI)	
	Model 1	**Model 2**
Midlife cholesterol		
Men		
< 6.5 mmol/l	1.0	1.0
≥ 6.5 mmol/l	**2.6 (1.0–7.4)**	**2.2 (1.0–6.2)**
Women		
< 6.5 mmol/l	1.0	1.0
≥ 6.5 mmol/l	**3.5 (1.4–8.7)**	**2.3 (1.0–6.5)**
Midlife systolic BP		
Men		
< 140 mmHg	1.0	1.0
140 - 159 mmHg	1.9 (0.5–6.5)	2.7 (0.7–10.8)
≥ 160 mmHg	**5.0 (1.5–17.1)**	**4.6 (1.1–19.2)**
Women		
< 140 mmHg	1.0	1.0
140 - 159 mmHg	2.2 (0.9 - 5.4)	1.9 (0.7 - 5.0)
≥ 160 mmHg	2.3 (0.9 - 6.2)	1.7 (0.6 - 4.9)

Multiple logistic regression analyses were used to create the models. Model 1 gives crude odds ratios (OR) with 95 % confidence intervals for AD, model 2 includes adjustments for age, apolipoprotein E (ε4/non-ε4), education, and body mass index. Significant results are marked with bold font. BP = blood pressure.

5.7.4 Combined effect of midlife cholesterol, systolic BP, and ApoE

Of the men with AD, 26 % possessed the combination of high midlife cholesterol and systolic BP and the ApoE ε4 allele compared to 5 % of the non-demented men ($p < 0.001$). Only 5 % of the men with AD had none of these three risk factors compared to 27 % of non-demented men. Of women with AD, 14 % had all these risk factors compared to only 4 % of non-demented women ($p = 0.014$). Of women with AD, 14 % also did not have any of these risk factors, while in non-demented women this proportion was 27 % (figure 4).

The combination of all these three risk factors, i.e. high midlife cholesterol, high midlife systolic BP, and ApoE ε4 allele, markedly increased the risk of AD in both men (OR = 21.1, 95 % CI 2.3-195.2) and women (OR = 5.4, 95% CI 1.1-25.9), after adjustments for age and education.

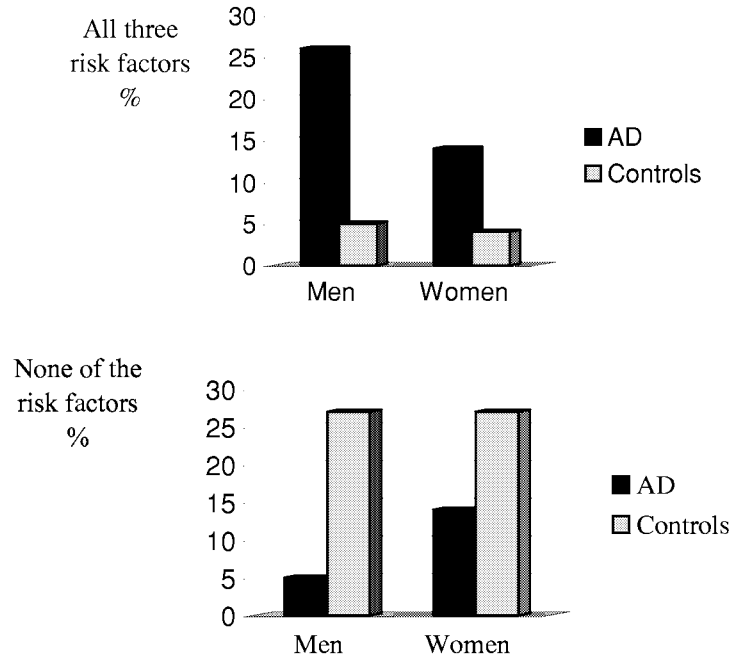

Figure 4. Association between Alzheimer's disease and midlife high cholesterol (≥6.5 mmol/l), high systolic blood pressure (BP ≥160 mmHg) and ApoE ε4 allele in men and women (all three risk factors vs. none of these risk factors).

5.7.5 Association between late-life vascular diseases and AD

The history of MI at late-life was equally common in men and women with AD. However, the history of MI was significantly associated with AD in women only (table 18). Similarly, the presence of cerebrovascular symptoms and diabetes mellitus at late-life were significantly associated with AD in women only, even after adjustments were made.

Table 18. Association between late-life vascular diseases and Alzheimer's disease in men and women.

Characteristic	Odds ratio (95 % CI)	
	Model 1	Model 2
Myocardial infarction		
Men	1.4 (0.8–2.4)	1.4 (0.6–3.7)
Women	**6.1 (2.7–13.8)**	**3.8 (1.5–9.7)**
Cerebrovascular symptoms		
Men	2.4 (0.7–8.6)	1.6 (0.3–8.1)
Women	**4.4 (1.7–11.4)**	**2.9 (1.0–8.8)**
Diabetes mellitus		
Men	0.8 (0.3–2.3)	0.8 (0.3–2.4)
Women	**2.0 (1.2–3.3)**	**1.8 (1.0–3.1)**

Multiple logistic regression analyses were used to create the models. Model 1 gives crude odds ratios (OR) with 95 % confidence intervals for AD, model 2 includes adjusting for age, apolipoprotein E (ε4/non-ε4), education, and body mass index. Significant results are marked with bold font.

5.7.6 Interactions between vascular risk factors and gender

The putative interactions between gender and the following factors: ApoE, midlife cholesterol, midlife systolic BP, the combination of these three risk factors, and MI, cerebrovascular symptoms, and diabetes mellitus (one at a time), were tested using logistic regression analyses. A significant interaction between gender and MI for the development of AD was found ($p = 0.005$), but no other significant interactions were seen.

5.8 Estrogen replacement therapy and the risk of MCI and AD (Study V)

5.8.1 Clinical characteristics according to ERT status

Altogether, 250 women (31 %) had received ERT and 550 (63 %) had not received ERT. The mean age of starting ERT was 50.5 (± 7.5) years, and the mean duration for the therapy was 8.9 (± 7.8) years.

Women who had not received ERT were older both at midlife (51 vs. 50 years, $p = 0.015$) and late-life assessments (72 vs. 70 years, $p < 0.001$) and they had fewer years of formal education (7.9 vs. 9.3 years, $p < 0.001$) than women that had received ERT. Women with no

ERT history also had higher BMI and higher serum total cholesterol at midlife (BMI: 27.0 vs. 25.5 kg/m^2, $p < 0.001$; cholesterol: 6.8 vs. 6.5 mmol/l, $p = 0.003$) and at late-life (BMI: 28.5 vs. 27.5 kg/m^2, $p = 0.004$; cholesterol: 6.1 vs. 5.9 mmol/l, $p = 0.005$) than women that had received ERT. Moreover, women that had not received ERT had higher systolic BP (146 vs. 141 mmHg, $p < 0.001$) and diastolic BP (89 vs. 87 mmHg, $p = 0.009$) at midlife, but at late-life there was no difference between the groups. There were no differences according to ERT status in the prevalence of MI or cerebrovascular symptoms at midlife or late-life. The distribution of ApoE ε4 allele carriers was similar in two groups.

5.8.2 Estrogen replacement therapy and MCI

Only 13.2 % of the women with MCI had used ERT compared to 33 % of the controls ($\chi^2 = 6.6$, $p = 0.01$). Accordingly, women that had received ERT had a significantly lower risk of MCI than non-users (crude OR = 0.31. 95 % CI 0.12-0.79). Adjusting for age, education, midlife cholesterol, midlife systolic BP, and late-life MI and cerebrovascular symptoms, and ApoE ε4 status did not change the results (adjusted OR = 0.39, 95 % CI 0.15–0.98). In addition, the results remained substantially similar when the MCI subjects with health problems that could have an impact on cognitive functions (n = 10) were included in the analyses (results not shown).

Because of the small number of women that had received ERT in the MCI group, the results were verified using logistic regression analysis with the LogXact program for Windows, v.4.0 (Cytel Software Corporation). This gave similar results to the main analyses.

5.8.3 Estrogen replacement therapy and AD

Only one single subject with AD (4.5 %) had a positive ERT history compared to 33 % of the controls ($\chi^2 = 7.9$, $p = 0.005$). Thus, women that had received ERT had a significantly lower risk of AD than non-users (crude OR = 0.10, 95 % CI 0.01-0.72). Importantly, multiple adjustments (as for MCI) did not change the association (adjusted OR = 0.10, 95 % CI 0.01-0.84). These results were also verified using logistic regression analysis with the LogXact, which gave similar results to the main analysis.

This single AD patient who had received ERT was of ApoE ε3/4 genotype. She had had elevated serum cholesterol level at midlife, had suffered from MI, and also displayed symptoms of cerebrovascular disease prior to the re-examination. She had used ERT for only two years, and had had a relatively early menopause at the age of 45 years.

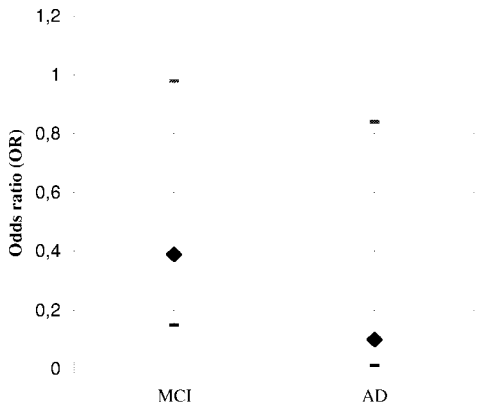

Figure 5. Estrogen replacement therapy and MCI and AD. Odds ratios (circles) and 95 % confidence intervals (horizontal lines) of the risk of MCI and AD in women that had received estrogen replacement therapy after adjusting for age, education, midlife cholesterol and systolic blood pressure, vascular diseases, and ApoE ε4 status.

6 DISCUSSION

6.1 Vascular risk factors and Alzheimer's disease

The present study showed that midlife elevated systolic BP and high serum cholesterol levels increased the risk of late-life AD. The combination of these risk factors at midlife increased the risk to a greater extent than either of the risk factors alone. These findings remained significant even after multiple adjustments. In contrast, elevated diastolic BP at midlife was not associated with an increased risk of AD in later life.

Why have many previous studies not been able to show an association between these vascular risk factors and AD? One potential reason may lie in the study setting. Most studies have been cross-sectional or have had relatively short follow-up times. BP and cholesterol values have been reported to decline before the manifestation of dementia (Skoog et al., 1996; Notkola et al., 1998; Guo et al., 2001), which is a finding also supported by our study. And AD is a catabolic state with low BP, low total cholesterol, and with low blood glucose concentrations (Landin et al., 1993).

6.1.1 Blood pressure as a risk factor for AD

The association between BP and AD is supported by two previous longitudinal, population-based studies which have reported a relationship between elevated BP earlier in life and the subsequent development of AD (Skoog et al., 1996; Launer et al., 2000). These studies, however, rather suggested that the association was between elevated diastolic BP and AD, in contrast to our finding of an association between elevated systolic BP and AD. Differences in the study settings and populations could account for these partly conflicting results. For example, the study of Skoog et al. examined the relationship between BP at old age (70 years) and the development of dementia later in life, whereas our study investigated the association between BP at midlife (mean age 50 years) and late-life AD. Age-related phenomena, such as duration of antihypertensive medication or changes in blood vessel structure, may modify the BP level in the elderly to the extent that it no longer reflects what may be the more long-term level of BP exposure. Therefore, our results may be a better reflection of the effect of long-lasting elevated BP on the development of AD.

Furthermore, treatment patterns may have partly contributed to our finding. In our study, subjects with AD were more likely to receive antihypertensive drug treatment at midlife than non-demented subjects. Yet, despite the treatment, subjects with AD still had higher systolic BP values at midlife than their non-demented counterparts. This is in line with the findings that a considerable proportion of treated hypertensive patients do not achieve target BP levels (Coca, 1998; Klungel et al., 2000). This could also reflect the fact that traditionally it has been diastolic BP, and not systolic BP, which has been the main indication for antihypertensive treatment (Applegate et al., 1989; Launer et al., 2000; Mancia et al., 2002). A recent study revealed that this is the situation even today by showing that systolic BP control by treatment was much less frequent than diastolic BP control (Mancia et al., 2002). Thus, it is possible that in our study, which included both treated and untreated subjects, the effect of medication could account for the observed results, and having reduced the risk of diastolic BP, the risk related with systolic BP became evident. It is noteworthy that in the Honolulu-Asia Aging Study (Launer et al., 2000), the elevated diastolic BP at midlife was predictive for AD only in subjects never treated with antihypertensive drugs. From this perspective, our data should not be interpreted as minimising the potential risk of AD in subjects with elevated diastolic BP, but rather as emphasising the importance of elevated systolic BP as a risk factor for AD, even in subjects with normal diastolic BP. Interestingly, in the Honolulu-Asia Aging Study, AD pathology was more strongly associated with midlife elevated systolic BP, although clinical AD was most strongly associated with diastolic BP (Petrovitch et al., 2000). The only trial showing that BP control can prevent dementia was carried out in patients with isolated systolic hypertension whose diastolic BP was normal (Forette et al., 1998), thus corroborating our findings that elevated systolic BP may be important in the development of AD.

It is known that with aging, the systolic component of BP tends to increase whereas diastolic BP remains the same or may even decrease (Franklin et al., 1997). Our results are in line with this; in the whole study sample, systolic BP increased during the follow-up whereas diastolic BP decreased. However, also systolic BP decreased during the follow-up among those who developed AD. Significant health outcomes associated with BP concentrate generally on systolic BP among the elderly. In the context of prevention of AD when focusing on BP earlier in life, the diastolic versus systolic BP enigma may not be of major

relevance because both elevated systolic and diastolic BP at midlife are currently considered to be risk factors for cerebrovascular and cardiovascular diseases, and should be treated. However, the relative importance of various components of BP in predicting the risk of cardiovascular disease is a subject of debate. During recent years, the focus has shifted from diastolic BP to systolic BP, and recently also to pulse pressure (Franklin, 1999; Deedwania, 2002). Regarding dementia and AD, there have been only few population-based studies specifically addressing the importance of different components of BP. Thus, this issue needs to be further studied in different age groups and at different time points in prospective population-based studies to better understand the relationships between BP and AD.

A recent longitudinal cohort study found little association between BP levels during the 15 years of observation and risk of AD (Morris MC et al., 2001). The inverse association between BP measured four years previously and risk of AD is puzzling, especially when there was lack of association between disease risk and BP measurements at other times. In that study, the BP measurements for the whole follow-up period were lacking in about one third of the study population, and quite few individuals had high BP levels. This may have resulted in wider confidence intervals, which also included the odds ratios reported in our study and in the study of Launer et al. (2000). Another explanation to this inverse finding could be related to the long-term effects of hypertension. It is possible that after a certain critical period when cerebrovascular pathology has already taken place, higher BP is needed to maintain an adequate cerebral perfusion.

In addition to these recent longitudinal studies on the risk of AD and hypertension, the first studies showing reduced incidence of AD in subjects treated with antihypertensive drugs have been recently published (Forette et al., 1998; Guo et al., 1999). However, some studies with antihypertensive agents have reported no protective effect on AD (SHEP 1991; in't Veld et al., 2001). This variation in results can be attributed to differences in study design, sample size, or the length of the follow-up. It must be noted that at present no trial has been designed specifically to prevent AD in hypertensive patients, and the results have been derived from secondary analyses only. Furthermore, one plausible reason for the modest effect of lowering BP in some observational studies may be that a considerable proportion of treated patients do not achieve the targeted BP levels. Studies from treatment of hypertension in Finland suggest that target levels are achieved in only about 20 to 40 % of hypertensive

subjects (Kastarinen et al., 1998; Marques-Vidal and Tuomilehto, 1997) and similar figures have been reported throughout the world (Coca, 1998). Diagnosis of hypertension, and even prescription of drugs to treat the condition, does not assure that the compliance to or efficacy of the treatment is adequate.

6.1.2 Cholesterol as a risk factor for AD

Our study revealed that high serum total cholesterol level at midlife was a risk factor of late-life AD. This finding is consistent with that reported from the Finnish cohort of the Seven Countries Study in elderly males (Notkola et al., 1998). Our study contained both males and females, and therefore, these data can be used to generalise the association between elevated midlife cholesterol levels and the development of AD. Furthermore, this confirmation of the previous findings in an independent and larger cohort indicates that this association is real.

Besides these two studies, there appear to be no other long-term (decade or more) prospective studies specifically addressing the association between cholesterol levels and development of dementia. There are some prospective studies with relatively short follow-up times yielding negative or conflicting results on the association between cholesterol and AD (Kuusisto et al., 1997; Romas et al., 1999; Slooter et al., 2000). There is evidence that cholesterol values gradually fall at later ages, and evidence of more rapid decrement in those destined to develop dementia (Jarvik et al., 1995; Notkola et al., 1998), as observed also in our study. Thus, temporal relation between risk factors and AD cannot be determined unequivocally in the studies with relatively short follow-up times, and this is probably why the association between high cholesterol and AD have been missed.

Consistent with our results, many studies in the elderly have not been able to show a relationship between cholesterol values and AD cross-sectionally (Hofman et al., 1997; Wieringa et al., 1997; Notkola et al., 1998). However, at least two autopsy studies have reported elevated serum total and LDL cholesterol in very old patients with AD (Kuo et al., 1998; Lesser et al., 2001). The latter of these was conducted among elderly of a single nursing home residents, and no control group was available, but AD patients were compared with non-AD dementia patients, making wider interpretation of the findings difficult. Furthermore, *post mortem* examination of an AD brain reflects only the last stage of a

progressive neurodegenerative process that started decades before, and at that stage, little information can be gained as to what initially started the disease process.

In the framework of the autopsy series in the Honolulu Asia Aging Study, the association between midlife cholesterol levels with NPs and NFTs was studied in a sample of 89 Japanese-American men (Launer et al., 2001). Interestingly, low midlife total cholesterol was associated with a lower number of neocortical NPs and NFTs, but late-life total cholesterol was not associated with the neuropathological markers. There was also a linear association for increasing midlife and late-life HDL cholesterol levels and an increasing number of NPs and NFTs (Launer et al., 2001). These results suggest that cholesterol and HDL may play a role in the formation of AD pathology. From a causal point of view, midlife cholesterol is interesting because increased cholesterol levels and the beginning of Aβ depositions appear to occur in parallel (Braak et al., 1995). Mechanisms of cholesterol action in the brain and factors associated with abnormal lipid metabolism in the development of AD and in the different phases of the diseases need to be better understood to fully interpret these results.

Our conclusion about the association between elevated cholesterol and AD is supported by recent studies showing reduced rates of AD in individuals who had used statins to reduce their serum cholesterol levels (Jick et al., 2000; Wolozin et al., 2000; Rockwood et al., 2002). There is also strong biological evidence and experimental studies supporting the association between cholesterol and AD as discussed later.

6.1.3 Vascular diseases and AD

Clinical indicators of atherosclerosis, such as previous MI and cerebrovascular symptoms, were found to be more frequent in individuals with AD than in non-demented individuals, suggesting that impaired cardiovascular and cerebrovascular function may increase the susceptibility to AD. Thus, our results are in line with the large population-based cross-sectional prevalence study from Rotterdam, which showed an increased risk of AD among persons having atherosclerosis (Hofman et al., 1997), and with another study having reported an association between MI and AD (Aronson et al., 1990). The increased prevalence of atherosclerosis among subjects who developed dementia can be considered to be a consequence of high cholesterol and BP values. Indicators of symptomatic atherosclerosis

are, however, the final stages of the atherogenetic process in the peripheral system. It is possible that hypertension and/or hypercholesterolemia act through different pathophysiological mechanisms to provoke dementia and vascular atherosclerosis. People with symptomatic atherosclerosis represent survivors of this disorder in the population, since both coronary heart disease and cerebrovascular disease have high mortality. Thus, any outcome such as dementia observed among survivors may be an underestimation of the true risk.

6.1.4 Possible mechanisms

There are many neuropathological mechanisms that could account for the association between hypertension and hypercholesterolemia and the development of AD, but the precise mechanisms remain unknown at the moment. Hypertension and/or hypercholesterolemia have been suggested to increase the risk of dementia, for instance by inducing atherosclerosis and impairing cerebral blood flow, but they may also directly induce AD neuropathology (Skoog et al., 1999 b). The addition of vascular events to our analyses did not change the association between midlife high systolic BP, cholesterol levels, and subsequent AD, suggesting that hypertension and hypercholesterolemia *per se* may also have a role in the pathogenesis of AD. Furthermore, the combination of hypertension and hypercholesterolemia at midlife was a particularly strong risk factor for AD. Thus, these factors may accelerate the development of AD at least partly through different pathophysiological mechanisms.

6.1.4.1 Hypertension and AD

Hypertension is a risk factor for white matter lesions and cerebrovascular disease, which are commonly found in dementia at old age (Skoog et al., 1996; Breteler et al., 1998; De Leeuw et al., 2002). Hypertension may contribute to these changes by altering the autoregulation of blood flow to the brain, or contributing to arteriosclerosis, hyalinoses, disturbed endothelial function, and brain cell permeability, or through cardio-emboli (Romas 1996; Farkas and Luiten, 2001). For WML, the following changes have been suggested to be of relevance: the adaptive changes in the cerebral circulation causes improved tolerance in increase in pressure, while acute hypotension may lead to cerebral ischaemia at blood pressure levels well tolerated by normotensive individuals. Thus, subsequent episodes of hypotension (e.g.

those induced by drugs or cardiac failure) may lead to hypoperfusion and ischaemia in vulnerable areas, such as in the deep white matter, which is supplied by long penetrating end-arteries which have few collaterals. The resulting demyelinisation might lead to dementia through disconnection of the subcortical-cortical pathways (Skoog et al., 1996). It has been demonstrated that WML are associated with impaired cognitive functions, in particular declined attention and speed of mental processing (Gunning-Dixon and Raz, 2000). WML have also been related to accelerated cognitive decline in individuals with MCI (Wolf et al., 2000). However, the clinical significance of WML is still a subject of debate.

Hypertension is also a risk factor also for stroke. There is evidence that stroke and AD occur in the same patient more frequently than would be anticipated by chance (Pasquier et al., 1998). It is possible that due to the summation of the various types of lesions, stroke, even though it has only minor physical manifestations, may lead to an increased progression towards cognitive decline in AD patients. In fact, the Nun Study reported that fewer neuropathological lesions of AD resulted in dementia in those individuals with lacunar infarcts (Snowdon et al., 1998), indicating that vascular lesions can influence the clinical expression of AD.

Hypertension may interfere with proper cerebral circulation and neuronal metabolism. Several studies have indicated that the cerebral capillary ultrastructure is damaged in AD, and the regional blood flow of AD patients is impaired compared with age-matched control individuals (Farkas and Luiten, 2000). Chronic hypertension can initiate general vasoconstriction resulting in decreased cerebral perfusion rate, which can give rise to microanatomical aberrations in the capillary ultrastructure of cortical areas (basement membrane thickening, perivascular collagen deposits). Consequently, capillary basement membrane pathology will either physically hinder passive nutrient trafficking from blood to brain or interfere with active transport processes (Farkas and Luiten, 2000). The neural compartments will thus be deprived of essential nutrients with a major focus on glucose, thus compromising their energy status and metabolic activity. This may result in neuronal dysfunction, which may further lead to structural neuronal disintegration and eventually to cell death and cognitive failure (Farkas and Luiten, 2000). The finding that Aβ interacts with endothelial cells producing vasoconstriction and superoxide-mediated endothelial damage may also be relevant in this context (Thomas et al., 1996; Thomas et al., 1997a; b).

Furthermore, endothelium participates in creating a blood brain barrier (BBB), and thus, hypertension-related pathological alterations in the endothelial structure may correspond with BBB failure such as transient leakage. BBB dysfunction has been suggested to be involved in the etiology and pathogenesis of AD (Claudio, 1996; DeJong et al., 1997; Skoog, 1997). These findings give support to the arguments that AD is a microvascular disorder with neurodegenerative consequences (de la Torre and Stefano, 2000; de la Torre, 2002).

Hypertension has also been related to increased brain atrophy (DeCarli et al., 1999; Petrovitch et al., 2000). In the National Heart, Lung, and Blood Institute Twin Study, elevated systolic and diastolic BP levels at midlife, an average of 25 years earlier, were inversely related to brain volumes at late-life (DeCarli et al., 1999). Furthermore, an increased amount of neuropathological markers of AD in individuals with elevated BP have been reported (Sparks et al., 1995; Petrovitch et al., 2000). These results indicate that in addition to accepted association of high BP with cerebrovascular lesions, there may be a direct relationship with neuropathological changes of AD.

6.1.4.2 Hypercholesterolemia and AD

There are many neurobiological theories that could explain why elevated cholesterol levels can lead to the development of AD. Firstly, longstanding hypercholesterolemia may lead to thickening of intima and weakening of endothelial functions in cerebrovascular arterioles and capillaries and these changes may impair brain metabolism (Levine et al., 1995). Secondly, cholesterol may also modulate APP metabolism. Several interesting experimental studies examining ways that cholesterol lowering might influence AD and APP metabolism (Fassbender et al., 2001; Kojro et al., 2001), further discussions on the issue (Haley, 2000; Wolozin, 2001), as well as a review on the relationship between cholesterol and the amyloid hypothesis (Hartmann, 2001; Simons et al., 2001) have been published recently. The finding that depletion of intraneuronal cholesterol inhibits Aβ production *in vitro* (Simons et al., 1998) and *in vivo* (Fassbender et al., 2001) are of interest. However, it could be speculated that this effect is due to some of the side effects of statins and not to the lowered cholesterol levels. The study of the Fassbender et al. (2001) compared the effects of statins and methyl-β-cyclodextrin (CDX), which physically extracts cholesterol from the plasma membrane. These are two very different models of action, but both of them were shown to lower

cholesterol and Aβ levels suggesting that it is really the reduction in cholesterol that caused decreased Aβ production in cell cultures.

The study by Fassenbender et al. (2001) is important because it shows that statins may reduce Aβ production also in a less artificial situation than *in vitro* experimentation. In guinea pigs, treatment with a high dose of statins induced a sharp decrease in Aβ levels in brain and CSF, and when the treatment ended, Aβ levels were restored, indicating no permanent damage to Aβ-producing systems (Fassbender et al., 2001). However, guinea pigs do not develop amyloid plaques, irrespective of treatment. Reduced Aβ42 levels will result in reduced or delayed plaque formation, but it is not known whether amyloid plaques or a soluble monomeric or olicomeric Aβ42 are the most important during early AD. Thus, the study by Refolo et al. is of importance, because it showed that actual plaque formation can be inhibited, and that the observed effects are not drug-specific; cholesterol synthesis inhibitor BM15.766, which acts at a later state of cholesterol synthesis than statins, reduced plaque formation in transgenic mice (Refolo et al., 2001). Taken together, these findings suggest that increased cholesterol levels could accelerate the production of Aβ, the accumulation of Aβ plaques, and the development of AD, whereas lowering of cholesterol could reduce or prevent these processes.

How might cholesterol act on Aβ-producing system? An interesting link was found recently; it was observed that inhibition of APP β-secretase occurs in parallel to cholesterol reduction (Simons et al., 1998). In contrast, the activity of the α-secretase is increased upon cholesterol reduction (Kojro et al., 2001). Thus, cholesterol reduction appears to modulate the major APP secretases in such a way that they switch from the amyloidogenic to the non-amyloidogenic pathway.

It should also be noted that blood cholesterol and brain cholesterol are in two separate pools, and therefore, studies measuring serum cholesterol observe only the tip of the iceberg (Hartmann, 2001). Recent studies showing reduced rates of AD in patients taking statins do not indicate whether this is due to reduction in plasma cholesterol levels, decreased cholesterol synthesis in the CNS, or some other mechanisms. In addition to reducing cholesterol levels, statins appear to have a variety of mechanisms of action which may be

beneficial for CNS and be associated with a reduced risk of AD, including endothelial protection via actions on the nitric oxide synthase system, and antioxidant, anti-inflammatory, anti-platelet effects, and immunomodulatory effects (Sarti et al., 2000; Cucchiara and Kasner, 2001). Interestingly, a recent study showed for the first time that simvastatin modifies cholesterol metabolism in the human brain, and was shown to reduce the level of plasma 24S-hydroxycholesterol, which is synthesised in the CNS. Furthermore, the percentage of reduction occurred independently of the reduction in total cholesterol, indicating that simvastatin reduces cholesterol turnover in the human brain (Locatelli et al., 2002).

6.2 Vascular risk factors and mild cognitive impairment

Our study found that high serum cholesterol levels at midlife increased the risk of MCI later in life. Furthermore, subjects with MCI tended to have higher systolic BP at midlife than control subjects, and the distribution of systolic BP values was also wider among MCI than control subjects, but, ultimately, high midlife systolic BP only approached significance as a risk for MCI. Of subjects with MCI, 61 % had had either elevated serum cholesterol or high systolic BP at midlife. Although the midlife cholesterol levels and systolic BP of the subjects with MCI were higher than the control subjects, they were less abnormal than values in those individuals who developed dementia. Thus, with respect to these midlife vascular risk factors, subjects with MCI appear to be distinct from and intermediate between subjects with dementia and cognitively normal individuals.

The MCADRC criteria for MCI excluded those subjects with health problems that may have an impact on cognitive function. However, to investigate significant health-related contributors to the diagnosis of MCI at the population level, we also included individuals with some health problems in the main analysis. There was no significant difference in the prevalence of cardiovascular or cerebrovascular diseases between persons with MCI and controls. Importantly, exclusion of other health problems did not diminish the association between midlife high cholesterol and late-life MCI and the tendency between midlife high systolic BP and late-life MCI, but rather strengthened the effect of these risk factors. If we consider that the group of persons with MCI remaining after exclusion of other health problems represents a more homogenous group that is predictive of AD, these findings

provide further support for the view that hypercholesterolemia earlier in life predicts the development of cognitive impairment. The role of high systolic BP remains somewhat controversial based on this study, but the results of earlier long-term prospective studies suggest that high BP is associated with the development of cognitive impairment (Elias et al., 1993; Launer et al., 1995; Carmelli et al., 1998; Kilander et al., 1998; DeCarli et al 2001). Even though midlife BP values did not differ significantly between the individuals with MCI and controls in the present study, the formers more often reported a diagnosis of hypertension at midlife, further supporting the association between these conditions.

Pre-existing cognitive impairment is one of the main predictive factors for dementia. Thus, it is important to try to identify the risk factors for cognitive impairment in order to prevent dementia. The concept of MCI is of special interest in that sense because MCI is considered to be a high-risk condition for AD. Besides, the concept of MCI does not rely solely on neuropsychological tests, but is a clinically identifiable category where both psychometric and clinical aspects are taken into account. Apart from the present study, there appear to be no studies specifically investigating the relationship between midlife BP and the development of MCI applying the proposed diagnostic criteria (Petersen et al., 2001 a; b). Thus, further studies are needed to clarify the risk factors for MCI more specifically.

6.3 ApoE and Alzheimer's disease

The present study revealed that the association between the ApoE ε4 allele and AD was not altered when adjusting for midlife cholesterol and BP, and late-life vascular disease. Similarly, the association between elevated midlife cholesterol and systolic BP, and late-life AD was not altered when adjusting for the ApoE ε4 allele. These findings indicate that the ApoE ε4 allele, and midlife high serum cholesterol and systolic BP are all independent risk factors for late-life AD. Those individuals burdened with the combination of all these three risk factors have a particularly high risk of developing AD. Furthermore, the history of MI at late-life, which can be considered as a consequence of elevated cholesterol and BP, was associated with the risk of AD, also independently of the ApoE ε4 allele.

ApoE polymorphism is known to affect plasma lipid levels. A recent review has suggested that it accounts for approximately 10 % of the variation in the serum cholesterol levels in the

population (Mahley and Rall, 2000). In our study, the presence of ApoE ε4 allele was associated with elevated serum cholesterol both at midlife and late-life, but the ε4 carriers had only 0.12–0.17 mmol/l higher levels of cholesterol compared to non-carriers; such a small difference is not likely to be of major clinical significance. In addition, the association between ApoE ε4 and AD did not change when adjusting for cholesterol, further supporting the view that potentially important genotype-specific effects of ApoE in the nervous system may not necessarily be reflected in blood lipid levels (Romas et al., 1999).

Apart from the minor association between ApoE polymorphism and serum cholesterol levels, we found no consistent relationship between ApoE and vascular risk factors or vascular disease in midlife or late-life. This is line with many other cohort studies among elderly populations (e.g. Kuusisto et al., 1995; Prince et al., 2000), but an association between the ApoE ε4 allele and coronary heart disease has been reported (Wilson et al., 1996). The association between ApoE and stroke appears to be more controversial in general. A meta-analysis of nine case-control studies reported an excess of the ApoE ε4 allele among patients with ischemic cerebrovascular disease (McCarron et al., 1999), but some later studies have not confirmed this association (Catto et al., 2000; Frikke-Schmidt et al., 2001), neither have any population-based studies (Kuusisto et al., 1995; Basun et al., 1996; Skoog et al., 1998). These differences may at least partly result from differences in the study population, sampling strategies, and definition of the outcome.

The earliest investigations of the expression of the ApoE genotype concentrated on its influence on lipid metabolism and atherogenesis, and thus it was proposed that the effect of ApoE on AD may be mediated through dyslipidemia and vascular disease (Jarvik et al., 1995; Hofman et al., 1997; Notkola et al., 1998). Our results lend no support to this hypothesis, but suggest that some effects of ApoE other than that on serum lipids contribute to the development of AD. The multiplicity of the roles of ApoE within the central nervous system are currently being unravelled, and the very mechanisms relating ApoE ε4 allele to AD remain to be clarified. However, the fact that the ApoE ε4 allele and vascular risk factors all independently contributed to the risk of AD, and that the combination of these risk factors further increased the risk in what appears to be dose-dependent manner, suggests that there are several independent processes contributing to AD. Our results indicate that midlife

elevated cholesterol and BP may have a role in the pathogenesis of AD, independent from ApoE ε4, and that the foundations for AD may already be established at midlife, which is in accordance with histopathological evidence (Braak et al., 1999). It is important to note that ApoE allele ε4 is not only a risk factor for AD, but perhaps rather generally related with general predisposition for adverse outcome after non-specific brain insults. Individuals carrying ApoE allele ε4 are at increased risk of various dementias, and are also more likely to have impaired recovery of less specific brain insults following conditions such as closed head injury (Teasdale et al., 1997) or intracerebral haemorrhage (Alberts et al., 1995). Thus, it is possible that ApoE ε4 related susceptibility for AD emerges only after the onset of the disease process, and may be related to increased vulnerability or impaired recovery following various insults to the brain which, based on these data, may be of vascular origin (figure 6).

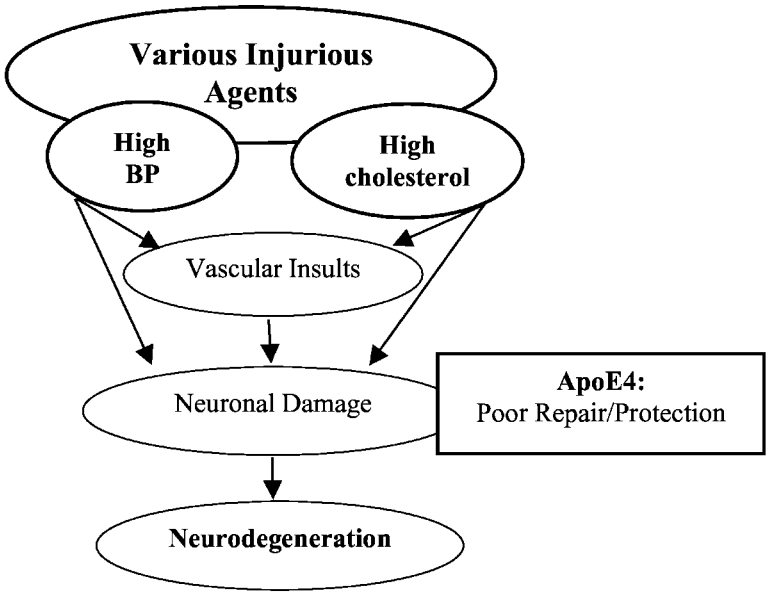

Figure 6. Possible processes for the development of AD

The fact that vascular risk factors seem to increase the risk of AD independently from ApoE genotype may have wide preventive implications. Causative mutations of AD are extremely rare, and the ApoE ε4 allele remains so far to be the only genetic risk factor for AD of established importance in the general population. The genetic constitution of an individual

cannot be changed, but there are effective means to reduce or prevent high cholesterol and BP levels. It is important to note that in this study the risk of AD related to the ApoE ε4 allele was only of a similar magnitude to the risk related to high midlife cholesterol levels or systolic BP, and it was substantially less compared to the risk of having both elevated cholesterol and systolic BP. Projecting from our data, effective interventions and good compliance to these interventions might, in terms of odds ratios, reduce the risk of AD from 8.2-11.0 to as low as 2.1, even if one was an ε4 carrier.

6.4 Gender-related factors and Alzheimer's disease

In this population-based study of persons aged 65 to 79 years, the prevalence of AD did not differ between men and women. Vascular factors were found to be associated with the risk of AD in both genders. However, the results suggested that there might be some differences between men and women in the contribution of various risks of AD. The main findings were 1) The effect of the ApoE ε4 allele as a risk factors for AD was observed to be more robust in men in this study population. Interestingly, the effect of the ApoE ε4 allele became stronger in both genders when restricted to those individuals that did not receive antihypertensive treatment at midlife. 2) High midlife cholesterol was a significant risk factor for AD in both genders. 3) High systolic BP at midlife was a significant risk factor for AD in men, but only approached significance as a risk factor in women. However, women more often received antihypertensive drug treatment at midlife than men, and women with AD were also more often treated with antihypertensive medications at midlife than those women who did not develop dementia, which may have reduced the risk associated with BP in women. 4) History of MI, cerebrovascular symptoms, and diabetes mellitus at late-life, which can be considered as a consequence of high cholesterol and BP, were significantly associated with AD in women only.

In our study, including subjects aged 65 to 79 years, there was no difference in the frequency of AD between men and women. A higher incidence rate of AD in women than in men has been described in some (Andersen et al., 1999; Fratiglioni et al., 2000 c), but not all studies (Bachman et al., 1993; Letenneur et al., 1994; Rocca et al., 1998; Ganguli et al., 2000). The discrepancies between the various studies may result from small sample sizes, particularly in the highest age range, use of different diagnostic criteria, and differences in participation

rates at follow-up. Our results are in accordance with recent findings from the large population-based Rotterdam Study, which used an extensive monitoring system so that virtually a complete follow-up of the cohort could be obtained, and reported no gender difference in the incidence of dementia up to a high age (Ruitenberg et al., 2001). In that study, the incidence of AD was higher for women only after 90 years of age.

We have shown that in men, both midlife elevated cholesterol and systolic BP significantly increased the risk of late-life AD. In women, midlife elevated cholesterol was a significant risk factor, and the effect of high midlife systolic BP approached significance. Women with AD had more often received antihypertensive treatment at midlife than their counterparts without dementia, supporting the association between the history of hypertension and AD also in women. Women also used antihypertensive drugs at midlife significantly more often and had lower diastolic BP values than men, i.e. were treated for hypertension earlier and more effectively than men. This is in line with the reports that women have a better awareness, treatment, and control status for hypertension than men (Marques-Vidal and Tuomilehto, 1997), and that diastolic BP has been traditionally the main target of treatment. Thus, it is possible that antihypertensive medication diminished the effect of BP on the risk of AD in women in our study. However, there may be different pathophysiologic effects of hypertension in women than in men (Rosenthal and Oparil, 2000). Therefore, it will be important to address possible gender differences in the effects of BP on the development of AD in future studies.

History of vascular diseases in late-life, i.e. survivors of MI and cerebrovascular disease, which can be considered as consequences of elevated cholesterol and BP, were significantly associated with AD in women, but not in men. Also in one earlier study reporting an association between a history of MI an AD, the observation was true only for women (Aronson et al., 1990). This may be due to selective survival, because men might be more likely to die from acute cardiovascular events during the follow-up than women. Surviving men may differ in many ways from the non-survivors and might possess properties protecting them against detrimental effects of vascular factors in the development of AD. These differences may also reflect the lack of statistical power in most studies; for instance, in our study, the sample size in men was relatively small, resulting in wide confidence intervals.

Among AD patients, the frequency of the ApoE ε4 allele was higher in men than in women Furthermore, the combination of the ApoE ε4 allele and midlife elevated cholesterol and systolic BP conferred a substantially greater risk of AD for men than for women. This suggests that in addition to these risk factors, women may have other factors modifying the risk for AD. Interestingly, we found that in the sub-population of persons not using antihypertensive drugs at midlife, the effect of ApoE was more pronounced, reaching statistical significance also in women even after multiple adjustments. Thus, the fact that women more often used antihypertensive drugs at midlife than men may have reduced the deleterious effect of ApoE in women. In the middle-aged populations, a large proportion of people with hypertension are untreated, and thus predisposed to the effects of high blood pressure. On the other hand, antihypertensive drugs may prevent vascular insults in the brain, and thus the limited capacity to repair neuronal damage linked to the ApoE ε4 allele might not become expressed in the treated group. Antihypertensive drugs may also have other beneficial effects for the CNS, which might protect against or diminish the detrimental effect of the ApoE ε4 allele. In the US Systolic Hypertension in the Elderly (SHEP) trial, with antihypertensive drug treatment based on diuretic chlortalidone as the first step and beta-blocker atenolol as the second step, the risk of stroke reduced significantly (SHEP 1991). The incidence of dementia did not, however, decrease. More recently, the Systolic Hypertension in Europe (Syst-Eur), using calcium-channel antagonist nitrendipine with ACE-inhibitor enalapril as a second step, confirmed that the risk of stroke in elderly patients with isolated systolic hypertension could be reduced (Staessen 1997). Moreover, in the Syst-Eur trial, the risk of dementia, particularly AD, was reduced in actively treated patients (Forette, 1999). Although direct comparisons between these two studies cannot be made, it is possible that calcium-channel antagonists may have some advantages regarding the prevention of dementia in hypertensive patients. On the other hand, the observational study by Guo et al. (2001) suggested positive effects, especially for diuretics. Thus, further studies are needed to clarify the effects of specific antihypertensive drugs.

Besides antihypertensive medication, there may be other environmental factors affecting the ApoE-related risk of AD in different genders. ERT might be one such factor. Sample sizes in our study were too small to directly analyse whether ERT modifies the ApoE-related risk of AD. The issue of ERT will be discussed more below. Another drug group of interest in the

context of prevention of AD is statins, which seem to have a variety of other mechanisms that may be beneficial for CNS in addition to the lipid-lowering effects, and may also be associated with a reduced risk of AD (Cucchiara et al., 2001). Statins have only been on the market in Finland only the mid 1990s. Thus, the usage of these drugs was not inquired about during the midlife assessment. Whether stains may diminish the ApoE-related risk of AD need to be specifically addressed in further observational studies and clinical trials.

6.5 Estrogen replacement therapy and AD and MCI

In the present study cohort including women aged 65-79 years, women who had used ERT had a significantly decreased risk of MCI and AD compared to those who had never received ERT. Women who had used ERT differed significantly from non-users with respect to age, education, cholesterol, and BP values; variables which are also associated with the risk of MCI and AD. Importantly, the protective effect of ERT for MCI and AD remained significant after controlling for these and other potentially confounding factors, supporting a biologically mediated association.

Previous studies examining the relationship between ERT and cognitive decline and cognitive impairment have yielded inconsistent results. For example, a recent study reported ERT to possess protective effects against cognitive decline in the Modified MMSE performance (Carlson et al., 2001), but in another study, ERT had no significant protective effect for cognitive decline or cognitive impairment in the Short Portable Mental Status Questionnaire performance (Fillenbaum et al., 2001). A problem also concerning research on ERT and cognitive impairment is that the outcome has not been uniformly defined across studies. To our knowledge, the present study is the first one to evaluate the association between ERT and MCI. Given that MCI is considered to be a high-risk condition for AD (Petersen et al., 2001), the observed inverse association between ERT and MCI could be taken as further evidence for the protective role of estrogen against cognitive impairment and the development of AD, and may point to the hypotheses that estrogen may influence the pathogenic processes of AD.

In the present study, the mean age of starting ERT was 51 years, thus near the menopause, and the mean duration of ERT use was 9 years. These results suggest that long-term estrogen

therapy, starting at midlife, displays a protective effect against the development of AD in late-life. It is possible that there is a certain critical period that takes place at menopause when withdrawal of hormonal influence renders the brain susceptible to pathological processes (Marder et al., 2000). ERT started during that time may have latent but most potent beneficial effects against AD. The data from the existing three larger-scale randomised placebo-controlled trials of ERT treatment in women with manifest AD have indicated that short-term (three to twelve months) ERT does not alter the progression of AD (Henderson et al., 2000; Mulnard et al., 2000; Wang et al., 2000). These findings in AD are analogous to findings from studies that have examined the effect of ERT in primary (Writing Group for the Women's Health Initiative Investigators, 2002) or secondary secondary prevention of cardiac disease (Hulley et al., 1998) or stroke (Viscoli et al., 2001), where ERT was reported to be ineffective. In an already damaged brain, estrogen treatment may be lacking in efficacy when potentially estrogen-responsive neurons have become refractory to the effects of estrogen or are already dead (Marder et al., 2000). Thus, ERT is likely to be more effective in preventing and delaying the onset of AD.

One shortcoming in the present study is the lack of specific information about the form of ERT used and the type of application procedures and doses. ERT preparations may differ in their ability to excerpt the putative protective effects. It would seem that all the intervention studies cited above used conjugated equine estrogens, a compounds that contain several forms of estrogen for which limited information is available regarding their neurobiological actions. This preparation has only been available in Finland in recent years. Consequently, considering the fact that the ERT treatments in our study date back several years, it is highly unlikely that many participants used this preparation of ERT. The prescribing of ERT in Finland has relied on 17β-estradiol, which has been suggested to be the most bioactive form of estrogen. Given the recent reports of adverse effects concerning the use of conjugated equine estrogens, the issue of which estrogen to use may be of great significance. Interestingly, a small randomised, double-blind, placebo-controlled trial using high-dose estradiol transdermally recently found a positive effect of estrogen on cognitive function (verbal and visual memory, and attention) in women with AD (Asthana et al., 2001). We assume that most of the subjects receiving ERT also received progestin, which may offset some of the effects of estrogen, most significantly in terms of raising the LDL and decreasing the HDL cholesterol levels in serum. In conclusion, regardless of the assumption that the

presence of progestin might oppose some of the beneficial effects of estrogen, the data still suggest protective effects of ERT for MCI and AD.

The grounds for initiation of the ERT therapy in the study participants are not known, but the age of initiation of ERT, average duration of ERT, and the praxis of ERT in Finland during that time, suggest that the main reason was climacteric symptoms. Prescription bias due to physicians starting ERT for cognitive impairment is highly unlikely, because cognitive impairment has not been an indication for ERT, nor is it even a current praxis. One limitation concerning self-reported information about ERT is that it may be less reliable in subjects with cognitive impairment. If women with cognitive decline were less likely to remember ERT use, it might cause a false-protective effect of ERT to be seen. The use of ERT was specifically inquired about in two separate questions in the self-administered questionnaire, which were sent to the participants' homes beforehand. In this case, a proxy informant (a spouse) could balance this potential shortcoming. Furthermore, compared to the reports concerning other medications, such as statins, antihypertensive drugs, and aspirin, no difference between the women with MCI or AD and the controls was observed (data not shown). Yet, we cannot entirely exclude the possibility that reporting bias had some effect on the results. There is also a possibility of selection bias due to non-participation. Those individuals who were lost during the follow-up were older, less educated, and displayed greater risks for vascular disease than those who participated; characteristics similar to those persons that had not used ERT. It is known that people with cognitive decline are less likely to participate in clinical studies (Launer et al., 1994). Thus, if non-participants were at an increased risk of cognitive impairment and dementia and possibly also used ERT less often, our results would represent an underestimation of the true effect of ERT rather than the opposite. Finally, because only five subjects that had received ERT developed MCI and only one subject developed AD, any stratified analyses by ERT status to examine whether ERT modifies the association between vascular risk factors, ApoE, and MCI or AD could not be conducted.

A strength for the ERT study was that we were able to control the analyses for a large number of potential confounding factors which constitute independent risk factors for AD, controlling for which did not change the association between ERT and decreased risk of MCI and AD. According to a recent review (LeBlanc et al., 2001), two previous cohort studies

(Tang et al., 1996; Kawas et al., 1997) were limited because of not assembling comparable groups. Also, in our study, there were significant differences in many clinical variables between those that had or had not used ERT, but we were able to adjust the analyses for the confounding effects of these variables.

In summary, our data present evidence that long-term postmenopausal ERT provides significant protection for late-life MCI and AD. Although observational studies cannot prove causal relations, they can be used as a guide in the interim before the results of ongoing randomised trials of ERT for preventing dementia (Shumaker et al., 1998; Wren et al., 1998) are available, which can take several years. While our results provide optimism about future prevention options for AD, ERT cannot currently be recommended as a general prophylactic measure against AD. ERT is widely used to control menopausal symptoms, and it has many potential benefits, including protection for osteoporosis and colorectal cancer. However, potential benefits of ERT must be individually weighted against the risks it may pose, including an increased risk of breast and endometrial cancer (Writing Group for the Women's Health Initiative Investigators, 2002). At the population level, routine ERT might decrease overall mortality in countries with high cardiovascular morbidity, but it might increase mortality in countries with lower cardiovascular morbidity (Panico et al., 2000). Ultimately, however, the decision of whether to prescribe ERT should be made on the individual level, with consideration of all the issues, including indicators for high risk of dementia. These results also warrant the need for future research to find out which forms of estrogen or estrogen receptor modulators would possess the most beneficial effects without significant health risks.

6.6 Methodological aspects

6.6.1 Study population and design

The present study has several strengths. The design of the study is a population-based, longitudinal study with a large cohort of participants and substantial response rate, which increases the reliability of the findings. We had information about the study subjects at midlife and late-life, with a mean follow-up time of 21 years. This study is the first long-term prospective study evaluating the effect of midlife blood pressure and cholesterol

simultaneously on the development of AD later in life in both males and females. We also used accurate diagnostic procedures, which are discussed more below.

However, some issues need to be addressed when interpreting the results of the present study. The possibility of selection bias due to non-participation was investigated in the non-participants. The prevalence of both elevated systolic BP and hypercholesterolemia at midlife was higher among persons that did not participant in the follow-up. According to earlier studies, people with cognitive decline are less likely to participate in clinical studies (Launer et al., 1994). Hence, if non-participants were at an increased risk of cognitive impairment and dementia, our results would represent an underestimation of the true effect of elevated systolic BP and cholesterol on the development of AD and MCI. It is also probable that, because of selection bias, our study participants had lower rates of dementia and MCI than the full population including non-participants.

There is also a possibility of selective survival related to ApoE polymorphism. The presence of the ApoE ε4 allele may increase cardiovascular mortality (Wilson et al., 1996). However, if this were indeed the case, our results again would tend to underestimate the risk related to ApoE ε4 and the vascular factors for the development of AD, due to the selective mortality.

6.6.2 Diagnoses

6.6.2.1 Alzheimer's disease

The prevalence rate of dementia among participants with mean age of 71 years in this population-based study was 4 %, which is in agreement with estimated prevalence rates (3 % for age group 70-74 years) (Fratiglioni and Rocca, 2001). A total of 57 participants were diagnosed as having dementia, of whom 48 fulfilled the diagnostic criteria for probable or possible AD, and accordingly, the prevalence of AD was 3.4 %.

Dementia was diagnosed according to internationally accepted diagnostic guidelines, including the DSM-IV criteria for dementia and the NINCDS-ADRDA criteria for AD. According to a recent report of the Quality Standard Subcommittee of the American Academy of Neurology concerning diagnosis of dementia (Knopman et al., 2001), the DSM

criteria for dementia have good reliability and should be used routinely. The NINCDS-ADRDA diagnostic criteria for AD were considered to have sufficient reliability and validity and were recommended for use.

We used a three-phase study protocol to diagnose dementia to increase the sensitivity and specificity of the diagnoses. However, as in the most epidemiological studies on AD, autopsy data were not available to ultimately confirm the clinical diagnosis. Nonetheless, the accuracy of the clinical diagnosis in our institute has been reported to reach 96 % for probable AD and 86 % for possible AD (Kosunen et al., 1996).

Nearly all of our dementia patients underwent brain imaging, mostly MRI. This is an important strength in our diagnostic procedure compared to many earlier population-based studies. All patients with AD showed generalised and mild to moderate medial temporal lobe atrophy. Furthermore, none of the AD patients showed appreciable vascular pathology on MRI scans. Isolated minor lacunae or periventricular white matter signal changes were not considered as exclusion criteria for AD. All AD patients scored four or less on the Hachinski ischaemia scale (Hachinski et al., 1975). However, we cannot totally exclude the possibility that some bias towards the diagnosis of degenerative dementias occurred, since some forms of dementia (e.g., subcortical vascular dementia), which are difficult to distinguish from AD on clinical grounds, may have been included in the AD group. Even though some concomitant vascular dementia pathology might have existed in the AD group, this does not change our conclusions; by altering BP and hyperlipidemia, it may be possible to reduce the risk of both AD and VaD related phenomena in the brain.

The issue of vascular pathology versus AD is, in fact, even more complicated if we take into account the fact that the very essence of AD is not yet fully determined. The significance of Aβ plaques or NFTs, the classic pathological features of AD, is not known (Joseph et al., 2001). The neuropathology of AD is suggested to extend beyond amyloid plaques and neurofibrillary tangles. More than 30 % of AD cases have been shown to exhibit cerebrovascular pathology, and certain vascular lesions such as cerebral amyloid angiopathy, microvascular degeneration, and periventricular white matter lesions are evident in almost all cases of AD (Kalaria and Ballard, 1999). It is equally intriguing that about one third of patients diagnosed with VaD will have AD-type pathology in autopsy (Kalaria and Ballard,

1999). There is evidence that stroke and AD occur in the same patients more frequently than would be anticipated by chance (Pasquier et al., 1998). This should be no surprise considering the assumption that AD and stroke may share the same risk factors, but should, instead, support our theory.

Taking into account the commonalties between AD and VaD, it might be stated that even the diagnostic criteria have their limitations. The commonly accepted diagnostic criteria for AD refuse the pathogenic participation of vascular factors *a priori*. However, there is no apparent pathophysiological reason to expect that cerebrovascular and Alzheimer's processes are mutually exclusive (De Iorio et. al., 1999). Indeed, this prejudice has been a limiting factor for the analysis of risk factors involved in AD; stroke is one of the diagnostic criteria for vascular dementia and generally excludes a diagnosis of AD, which may result in negative associations between risk factors for stroke and AD. Furthermore, the lack of biological markers for AD and VaD, the controversy regarding the definition of VaD, and the increasing evidence of vascular risk factors for AD suggest that the traditional differentiation between AD and VaD is no longer very clear (Aguero-Torres and Winblad, 2000).

6.6.2.2 Other dementias

Few other dementia forms, such as VaD and FTD were found in this population-based sample. Memory disorders are not necessarily a part of the initial presentation of these disorders, and thus they may be under-diagnosed when using screening (MMSE) and diagnostic instruments largely based on the concept of AD. Conventional concepts of dementia have been criticised to be "Alzheimerised" and there has been discussion concerning whether memory disorder should be a required part of the definition of dementia.

The different criteria for VaD, including NINDS-AIREN used in our study, have been reported to have low sensitivity, but higher specificity (Knopman et al., 2001). The NINDS-AIREN criteria are currently the most widely used in clinical drug trials on VaD, despite their limitations (Erkinjuntti, 2000). In general, it is now known that the current criteria for VaD select etiologically and clinically heterogeneous groups (Erkinjuntti, 2002). The Hachinski Ischemic Score (HIS) has been reported to have both high sensitivity and specificity (Moroney et al., 1997). While lacking neuroimaging criteria, HIS has been

suggested to be more suitable for identifying the majority of dementia patients with vascular dementia, i.e., those with at least some cerebrovascular pathology. Even though we used HIS to support the diagnosis of VaD in our study, VaD was diagnosed using the NINDS-AIREN criteria, and thus, the true prevalence of VaD in this study population is probably higher than we could detect.

We used the consensus guidelines when diagnosing the other types of dementias (FTD, LBD) (Neary et al., 1998; McKeith et al., 1996). These criteria have also been reported to have their limitations, particularly low sensitivity. All considered, there has been discussion about the need to refine the definitions of the common causing dementias (Knopman et al., 2001).

6.6.2.3 Mild cognitive impairment

By applying the MCADRC criteria (Smith et al., 1996), we found a 6.1 % prevalence of MCI in subjects aged 65 to 79 years in this population-based study. If memory tests were used as the only neuropsychological criteria for the diagnosis of MCI, the prevalence was 5.6 %. After excluding other health problems that may have had a impact on cognitive function, the prevalence of MCI was 4.8 %. These figures are in agreement with another recent population-based study in the same district, including subjects aged 60-76 years. In that study, prevalence of the amnestic form of MCI was 5.3 %. If tests of other cognitive domains than memory were also used for the diagnosis, the prevalence of MCI was 6.5 % (Hänninen et al., 2002). The prevalence of MCI was estimated to be 3.2 % in the Eugeria Project in France, including individuals aged 60 years and older (Ritchie et al., 2001). That study used strict criteria for MCI, excluding subjects with a deficit in any other area of cognition than in memory. One problem with the concept of MCI, however, still is the heterogeneous use of the term across different studies.

Our study was primarily focused on the detection of dementia, and thus only those subjects scoring ≤ 24 on the MMSE in the screening phase underwent the exhaustive examinations needed for the diagnosis of dementia and MCI. Thus, it is likely that some instances of MCI may have escaped detection at this threshold, and thus, the present study may underestimate the true prevalence of MCI in this population.

6.6.3 Assessment of risk factors and confounders

The survey methods were carefully standardised and comply with most international recommendations (Vartiainen et al., 1991). Vascular risk factors of interest were measured at midlife, when the levels are less influenced by preclinical diseases, comorbidity, or treatment. Thus, the midlife measurements are considered to better reflect the long-term exposure for these risk factors. Because AD changes may appear already 20–30 years before the manifestation of dementia (Braak et al., 1999), identification of risk factors at midlife may help in detecting true causal risk factors for the disease.

The protocol included only a single BP measurement at midlife for most of the participants. However, we have no reason hypothesise that those who ultimately developed AD would have been more likely to have falsely elevated BP values. We also carried out the analyses in only those in whom BP was measured twice (n = 571), which did not change the main results (data not shown). Furthermore, BP and cholesterol values were measured only during the midlife and late-life assessments, and thus, we were not able to account for changes in these variables over time with or without therapy. However, if interventions or drug treatment would influence these results, they would be likely to lead to reduced odds ratios, i.e. underestimate the observed risk rather than overestimate (Launer et al., 2000).

Cholesterol values were measured as non-fasting values. Non-fasting values are often used in epidemiological studies, mainly for practical reasons and also because non-fasting values are not significantly different from fasting levels for an individual (Bachorik et al., 1991). Thus, any variation in levels caused by these variables would be equally distributed between all subjects, and would not be expected to change the association found.

Information about vascular events was obtained from self-administered questionnaires, which may introduce some reporting bias. On the other hand, a proxy informant (spouse, other relative) may increase the reliability, and also, significant medical events such as MI are likely to be remembered.

6.6.4 Statistical analyses

We used serum cholesterol and BP primarily as categorical variables in the analyses. The categories used are in accordance with guidelines recommended for elevated cholesterol levels and high BP categories proposed for clinical interventions by various international task forces. Thus, our data can be directly compared to these clinical action levels. The approach of using categorical variables has also been widely used in earlier epidemiological studies. The use of categories in logistic regression analysis gives odds ratios from which comparable estimates of the magnitude of the association can be obtained. Furthermore, it is not known whether the association between these variables are linear, curvilinear, or if there is a threshold effect. To sort out these question would require quite sophisticated statistical analyses and probably also larger sample sizes than our present sample size. However, the association between midlife elevated cholesterol and BP and AD later in life remained significant when cholesterol and BP values were evaluated as continuous variables in our study (results not shown).

7 SUMMARY AND CONCLUSIONS

The aim of our study was to clarify the epidemiology of AD in a large, longitudinal population-based study sample with specific focus on the role of modifiable midlife vascular risk factors, hypertension, and hypercholesterolemia. The role of vascular factors in the development of MCI was also evaluated. In addition, the putative gender-related differences in the risk factor profiles of AD were evaluated, as well as the association between ERT and AD and MCI. From the results of these studies the following conclusions can be drawn:

1. Elevated systolic BP and high serum cholesterol levels, and particularly the combination of these risk factors in midlife increased the risk of late-life AD. These findings remained significant after controlling for a number of potential confounding factors. The observed relation between midlife vascular risk factors and AD may have implications for the prevention of dementia as both hypertension and hypercholesterolemia can be treated.

2. Midlife elevated serum cholesterol was also a significant risk factor for late-life MCI, with the effect of elevated systolic BP approaching significance. These data point to a role of midlife vascular risk factors in the development of late-life MCI.

3. Systolic BP values declined during the average follow-up period of 21 years in individuals diagnosed with AD whereas the values increased in individuals with MCI and in controls. Decrease in cholesterol values was more rapid among individuals with AD and MCI than in controls. At the time of the diagnoses, there was no significant difference in these values between the three groups. These changes may explain at least partly the inconsistent / negative results from the earlier cross-sectional or short-term follow-up studies on this issue. These findings give further support to the view that the temporal relation between risk factors and AD cannot be determined unequivocally in studies with relatively short follow-up times.

4. The ApoE ε4 allele, elevated midlife cholesterol, and high midlife systolic BP constitute independent risk factors for AD. The association between ApoE ε4 allele and AD does not seem to be mediated by vascular risk factors. The risk related to treatable risk factors – elevated cholesterol and BP – appears to be greater than the risk related to the ApoE

ε4 allele. Projecting from the data, effective interventions to these might, in terms of ORs, may reduce the risk of AD from 8.4-11.0 to as low as 2.1, even if one was an ε4 carrier.

5. Vascular risk factors are important in the development of AD in both genders, but there may be some gender-related differences in the risk factor profiles of AD. It also seems possible that early and effective antihypertensive drug therapy may protect against / diminish the detrimental effects of the ApoE ε4 allele in the development of AD.

6. The data present evidence on behalf of long-term postmenopausal ERT to provide protection for late-life MCI and AD and support the view that estrogen may be one important factor accounting for the gender differences in the risk of AD.

The conclusion that elevated BP and serum cholesterol levels may have an important role in the development AD is supported by some other long-term prospective studies, and studies which have reported a decreased incidence or prevalence of AD in persons receiving antihypertensive or lipid-lowering drug treatments as well as by experimental studies. Together, these data suggest that there may be a true causal relationship between these proposed risk factors and AD. It is important to notice that also many of the other proposed risk factors for AD (e.g. diabetes, smoking) may be vascular related.

At the moment we do not know the exact mechanisms concerning how elevated BP and cholesterol increase the risk of AD, nor what their relative importance in the pathogenesis of AD is. Nevertheless, the available data, including the data obtained from these studies, can be considered as convincing evidence to emphasise the need for clinical interventions to control these risk factors more effectively. Early interventions aimed at reducing these cardiovascular risk factors may have a profound impact on the future incidence and prevalence of AD. Best of all, there are already clear indications and a number of means available for treatment of hypertension and hyperlipidemia. Proper treatment not only increases the chances of escaping cardiovascular morbidity and mortality, but may increase the chances of eluding AD as well.

The finding that the risk related to modifiable vascular factors appeared to be greater than the risk associated with the ApoE ε4 allele, the most important genetic risk factor for AD so far,

gives further reason for optimism about future prevention strategies for AD. Moreover, the effect of the ApoE ε4 allele may not be uniform but it may be modified for instance by antihypertensive drugs. Finally, it would seem that ERT has a protective effect against MCI and AD. Thus, these data undermine the fatalistic attitudes toward AD and provide us with evidence for possible ways to try to reduce the risk and future burden of AD.

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APPENDIX: ORIGINAL PUBLICATIONS (I-V)