GENETICALLY DETERMINED HYPOMETABOLISM IN ALZHEIMER'S DISEASE AND MIDLIFE RISK FACTORS FOR COGNITIVE IMPAIRMENT

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"Vanhuuden tullessa mieli pakenee linnun tavoin lapsuuden päiviin."

– Sinuhe Egyptiläinen (Mika Waltari)

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ABSTRACT

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GENETICALLY DETERMINED HYPOMETABOLISM IN ALZHEIMER'S DISEASE AND MIDLIFE RISK FACTORS FOR COGNITIVE IMPAIRMENT

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The role of genetic factors in the pathogenesis of Alzheimer's disease (AD) is not completely understood. In order to improve this understanding, the cerebral glucose metabolism of seven monozygotic and nine dizygotic twin pairs discordant for AD was compared to that of 13 unrelated controls using positron emission tomography (PET). Traditional region of interest analysis revealed no differences between the non-demented dizygotic co-twins and controls. In contrast, in voxel-level and automated region of interest analyses, the non-demented monozygotic co-twins displayed a lower metabolic rate in temporal and parietal cortices as well as in subcortical grey matter structures when compared to controls. Again, no reductions were seen in the non-demented dizygotic co-twins. The reductions seen in the non-demented monozygotic co-twins may indicate a higher genetically mediated risk of AD or genetically mediated hypometabolism possibly rendering them more vulnerable to AD pathogenesis.

With no disease modifying treatment available for AD, prevention of dementia is of the utmost importance. A total of 2 165 at least 65 years old twins of the Finnish Twin Cohort with questionnaire data from 1981 participated in a validated telephone interview assessing cognitive function between 1999 and 2007. Those subjects reporting heavy alcohol drinking in 1981 had an elevated cognitive impairment risk over 20 years later compared to light drinkers. In addition, binge drinking was associated with an increased risk even when total alcohol consumption was controlled for, suggesting that binge drinking is an independent risk factor for cognitive impairment. When compared to light drinkers, also non-drinkers had an increased risk of cognitive impairment.

Midlife hypertension, obesity and low leisure time physical activity but not hypercholesterolemia were significant risk factors for cognitive impairment. The accumulation of risk factors increased cognitive impairment risk in an additive manner. A previously postulated dementia risk score based on midlife demographic and cardiovascular factors was validated. The risk score was found to well predict cognitive impairment risk, and cognitive impairment risk increased significantly as the score became higher. However, the risk score is not accurate enough for use in the clinic without further testing.

Keywords: cognitive impairment/dementia, Alzheimer's disease, [^{18}F]FDG-PET, twins, risk factors in epidemiology, cohort studies

TIIVISTELMÄ

Jyri J. Virta

PERINNÖLLISESTI MÄÄRÄYTYNYT HYPOMETALIA ALZHEIMERIN TAUDISSA JA KESKI-IÄN RISKITEKIJÄT KOGNITIIVISELLE HEIKENTYMISELLE

Perintötekijöiden merkitystä Alzheimerin taudin (AT) patogeneesissa ei tunneta täydellisesti. Perintötekijöiden merkityksen selvittämiseksi tutkimme seitsemän monotsygoottisen ja yhdeksän ditsygoottisen AT:n suhteen diskordantin kaksosparin sekä 13 terveen verrokin aivojen glukoosimetaboliaa positroniemissiotomografialla (PET). Perinteisessä mielenkiintoalueanalyysissä emme havainneet eroja verrokkien ja dementoitumattomien ditsygoottikaksosparikkien välillä. Sen sijaan vokselitasoisessa analyysissä ja automatisoidussa mielenkiintoalueanalyysissä dementoitumattomien monotsygoottikaksosparikkien aineenvaihdunta oli verrokkeja hitaampaa ohimo- ja päälaenlohkon aivokuorella sekä aivokuoren alaisissa tumakkeissa. Näissäkään analyyseissä emme havainneet eroja dementoitumattomien ditsygoottikaksosparikkien ja verrokkien välillä. Dementoitumattomien monotsygoottikaksosparikkien hypometabolia saattaa olla merkki lisääntyneestä AT:n perinnöllisestä riskistä tai perinnöllisesti määräytyvästä aivojen hypometaboliasta, joka puolestaan mahdollisesti altistaa AT:lle.

AT:n taudinkulkua estävää tai hidastavaa lääkehoitoa ei toistaiseksi ole tarjolla, joten taudin ehkäisy on erityisen tärkeää. Yhteensä 2165 suomalaista kaksosta osallistui kognitiivisia toimintoja arvioivaan puhelinhaastatteluun vuosien 1999–2007 aikana. Yhdistämällä haastattelutiedot vuoden 1981 kyselylomaketietoihin pystyimme arvioimaan keski-iän riskitekijöiden vaikutusta kognitiivisen heikentymisen riskiin. Suositukset ylittävä alkoholin kokonaiskulutus sekä raittius keski-iässä olivat riskitekijöitä myöhemmälle kognitiiviselle heikentymiselle verrattuna kohtuukulutukseen. Myös suurien alkoholimäärien juominen kerralla lisäsi riskiä kokonaiskulutuksesta riippumatta.

Korkea verenpaine, ylipaino sekä vähäinen vapaa-ajanliikunta keski-iässä lisäsivät kognitiivisen heikentymisen riskiä. Sen sijaan korkeaan kolesterolitasoon ei liittynyt suurentunutta riskiä. Riskitekijöiden kasaantuminen lisäsi riskiä entisestään, ja aiemmin kehitetty riskipisteytys ennusti kognitiivisen heikentymisen riskiä hyvin. Pisteytys ei kuitenkaan ole sellaisenaan riittävän tarkka kliiniseen käyttöön.

Avainsanat: kognitiivinen heikentyminen/dementia, Alzheimerin tauti, [^{18}F] FDG-PET, kaksoset, riskitekijät epidemiologiassa, kohorttitutkimus

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ABBREVIATIONS

AD	Alzheimer's disease
ANOVA	Analysis of variance
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
AUC	Area under curve
Aβ	Amyloid beta
BA	Brodmann area
BMI	Body mass index
CAIDE	Cardiovascular Risk Factors, Aging and Dementia
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
CI	Confidence interval
CSF	Cerebrospinal fluid
DLB	Dementia with Lewy bodies
DSM-III-R	Diagnostic and Statistical Manual of Mental Disorders, revised third edition
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, fourth edition
DZ	Dizygotic
fAD	Familial Alzheimer's disease
FDG	2-deoxy-2-[^{18}F]fluoro-D-glucose
FTD	Frontotemporal dementia
FTLD	Frontotemporal lobar degeneration
GMR	Glucose metabolic rate
HAAS	The Honolulu-Asia Aging Study
HR	Hazard ratio
ICD-10	International Classification of Diseases and Related Health Problems, 10th revision
LOR	Line of response
MCI	Mild cognitive impairment
MET	Activity metabolic equivalent
MICF	Mild impairment in cognitive function
MIRAGE	Multi-Institutional Research in Alzheimer's Genetic Epidemiology
MMSE	Mini Mental State Examination
MNI	Montreal Neurological Institute
MRI	Magnetic resonance imaging
MZ	Monozygotic
NFT	Neurofibrillary tangle
NIA-RI 1997	National Institute on Aging and Reagan Institute recommendations from 1997
NINCDS-ADRDA	National Institute of Neurological Disorders and Stroke –

	Alzheimer's Disease and Related Disorders
NINDS-AIREN	Internationale pour la Recherche et l'Enseignement en Neurosciences
OR	Odds ratio
PAQUID	Personnes Agées QUID
PET	Positron emission tomography
PIB	$[^{11}C]$6-OH-benzothiazole-1 or $[^{11}C]$Pittsburgh Compound B
PSEN-1	Presenilin-1
PSEN-2	Presenilin-2
p-tau	Phosphorylated tau protein
rGMR	Regional glucose metabolic rate
ROC	Receiver-operating characteristic
ROI	Region of interest
RR	Relative risk ratio
SD	Standard deviation
SIVD	Subcortical ischaemic vascular dementia
SP	Senile plaque
SPM	Statistical parametric mapping
VaD	Vascular dementia
VCI	Vascular cognitive impairment

LIST OF ORIGINAL PUBLICATIONS

I   Virta JJ, Karrasch M, Kaprio J, Koskenvuo M, Räihä I, Viljanen T, Rinne JO. Cerebral glucose metabolism in dizygotic twin pairs discordant for Alzheimer's disease. Dement Geriatr Cogn Disord. 2008; 25(1):9-16.

II   Virta JJ, Aalto S, Järvenpää T, Karrasch M, Kaprio J, Koskenvuo M, Räihä I, Viljanen T, Rinne JO. Voxel-based analysis of cerebral glucose metabolism in mono- and dizygotic twins discordant for Alzheimer disease. J Neurol Neurosurg Psychiatry. 2009; 80(3):259-66.

III   Virta JJ, Järvenpää T, Heikkilä K, Perola M, Koskenvuo M, Räihä I, Rinne JO, Kaprio J. Midlife alcohol consumption and later risk of cognitive impairment: a twin follow-up study. J Alzheimer's Dis. 2010; 22(3):939-48.

IV   Virta JJ, Heikkilä K, Perola M, Koskenvuo M, Räihä I, Rinne JO, Kaprio J. Midlife cardiovascular risk factors and a risk score as predictors of cognitive impairment. Submitted.

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1. INTRODUCTION

The age structures of countries all around the world are changing as the proportion of elderly people steadily increases (Department of Economic and Social Affairs 2005). Since age is the most important risk factor for dementia and Alzheimer's disease (AD), the medical, social and economic importance of dementia disorders is likely to increase. At present, pharmacological treatment of AD provides only limited symptomatic relief and does not alter disease progression (Birks 2006; McShane et al. 2006). Additionally, the pathogenesis of AD and especially the importance of genetic factors in the disease process are not fully understood. Therefore, it is important to clarify the pathophysiology of AD, and also to identify significant, possibly modifiable, early risk factors for cognitive impairment.

The macroscopic pathologic findings of AD include generalized atrophy affecting especially the frontal and temporomesial lobes. The microscopic neuropathology consists of neuritic or senile plaques (SP) and neurofibrillary tangles (NFT), and their appearance is considered to precede symptom onset by 10-15 years (Perrin et al. 2009). The most severely affected cognitive domain in AD is episodic memory throughout its clinical course.

AD patients have significant but anatomically restricted reductions in cerebral glucose metabolism which can be assessed using positron emission tomography (PET) (Herholz 2003; Herholz et al. 2007). PET can even hint at the presence of AD pathology *in vivo* in very early and presymptomatic AD (Chetelat et al. 2003; Minoshima et al. 1997), and there is some evidence that individuals with an elevated genetically mediated risk of AD have an altered cerebral metabolic rate (Järvenpää et al. 2003; Small et al. 2000).

Finnish twin pairs have participated in the Finnish Twin Cohort, and extensive knowledge about their characteristics has been collected i.e. in the form of follow-up questionnaires and register follow-up (Kaprio 2006; Kaprio and Koskenvuo 2002). Because of their unique genetic and familial characteristics, twins are valuable in gaining an understanding on the impact of genetic factors in traits or diseases. Additionally, twin cohort studies provide a powerful means of control for genetic and familial factors in epidemiological risk factor studies.

The present work assessed cerebral glucose metabolism in monozygotic (MZ) and dizygotic (DZ) twin pairs discordant for AD in order to better understand the genetic mechanisms of AD pathogenesis and/or genetically mediated changes in cerebral glucose metabolic rate (studies **I** and **II**). The second goal of the work was to assess whether midlife alcohol consumption, drinking patterns and multiple cardiovascular risk factors are determinants of a cognitive impairment risk over 20 years later in Finnish twins (studies **III** and **IV**). Because of the twin design, interactions between environmental and genetic or familial factors could be studied.

2. REVIEW OF THE LITERATURE

2.1 Dementia

2.1.1 Definition, prevalence and incidence of dementia

The 10th revision of the International Classification of Diseases and Related Health Problems (ICD-10) defines dementia as a syndrome due to a disease of the brain, usually of chronic or progressive in nature, in which there is a disturbance in multiple higher cortical functions, including memory, thinking, orientation, comprehension, calculation, learning capacity, language and judgment. Consciousness is not clouded. Impairments of cognitive function are commonly accompanied, and occasionally preceded by deterioration in emotional control, social behavior or motivation (World Health Organization 2010). The dementia criteria in the fourth edition of Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) require the development of multiple cognitive deficits manifested by impairment in memory and at least one other cognitive domain. The cognitive deficits must also cause significant impairment in social or occupational functions, and represent a significant decline from a previous level of functioning. (American Psychiatric Association 1994) Multiple clinically distinguishable disorders can be responsible for the syndrome of dementia, of which AD is the most common. Other causes include vascular dementia (VaD), dementia with Lewy bodies (DLB), frontotemporal dementia (FTD) and dementia of Parkinson's disease.

Mild cognitive impairment (MCI) was originally described as a subjective memory complaint with abnormal memory for age, but with normal activities of daily living, normal general cognitive function and no dementia. (Petersen et al. 1999) As MCI often represents the earliest clinical phase of AD, the diagnostic criteria were revised along with the diagnostic criteria for AD (see chapter 2.1.2.5). The new criteria define MCI as concern regarding a change in cognition with evidence of impairment in one or more cognitive domains. Independence in functional abilities is preserved and the criteria of dementia are not met. (Albert et al. 2011)

In a collaborative study of population-based cohorts including studies from eight European countries, the age-standardized prevalence of clinically diagnosed dementia was estimated to be 6.4 % in subjects at least 65 years old. The corresponding prevalences of AD and VaD are 4.4 % and 1.6 %, respectively, making them the two most common causes of dementia. The age specific prevalence of dementia increases with advancing age, i.e. the prevalence is 0.8 % in 65 to 69-year-old subjects but 28.5 % in those at least 90 years old. The prevalences of both AD and VaD show similarly increasing prevalences with advancing age. Dementia and AD are more common in women, with the difference from men increasing with advancing age. The prevalence of VaD is greater in men than women among those under 85 years of age. However, the sex differences might be attributable to survival differences between men and women caused by women's greater longevity. (Lobo et al. 2000) The above collaborative study included a Finnish population, and the prevalence of dementia in this population did not

differ significantly from the estimated mean prevalence. In addition, the proportion of AD cases was in accordance with other included populations. Previously, the prevalence of severe dementia has been estimated to be 6.7 % in the Finnish population aged 65 years and over (Sulkava et al. 1985).

In a similar collaborative study on the incidence of dementia with patients originating from seven European countries, AD accounted for 60-70 % and VaD for 15-20 % of all dementia cases. The incidence rates of all dementia and AD are higher in women, but in VaD no sex difference is seen. The incidences of dementia and AD increase exponentially with age in women, but in men they plateau after the age of 80. (Fratiglioni et al. 2000)

Post-mortem neuropathology studies also support the belief that AD is the most common cause of dementia. An autopsy cohort of over 1 000 demented individuals revealed that 86 % of the subjects had AD-related pathology, 7.3 % had VaD and 5.7 % had other disorders. However, only 42.8 % had 'pure' classical AD, while in a significant proportion of subjects AD pathology was associated with either cerebrovascular (22.6 %) or Lewy body pathology (10.8 %). (Jellinger 2006) In a sample of Finnish individuals aged 85 or more, 42 % of subjects with clinically diagnosed VaD or other dementia had neuropathologic AD, when individuals with progressive dementia together with stroke were considered to have VaD (Polvikoski et al. 2001). These findings emphasize the fact that the coexistence of both AD and cerebrovascular pathology is not unusual.

The prevalence and incidence estimates of AD and VaD described above are based on criteria that require cognitive impairment severe enough to fulfill the criteria of dementia. However, as diagnostic methods become ever more sophisticated it will most likely be possible to diagnose the neuropathological processes of these cognitive disorders before dementia develops, which can affect the prevalence and incidence estimates.

2.1.2 Alzheimer's disease

2.1.2.1 Pathology and pathogenesis

Generalized atrophy is seen in AD, although the temporomesial and frontal lobes are most severely affected. The cortical ribbon is particularly thin in the frontal and entorhinal cortices as well as in hippocampus. In addition, gross brain volume is diminished, and convolutions become atrophied, sulci widened, lateral ventricles enlarged and white matter reduced. There is also progressive loss of synaptic connections between neurons, and a loss of neurons in neocortical areas and hippocampus. The histological hallmarks of AD are SPs and NFTs, even though they are not completely specific for AD and are seen in normal aging as well (Price and Morris 1999). SPs are extracellular structures with three morphologic forms (diffuse, classic and burnt-out). The classic plaques have a dense amyloid core with amyloid beta (Aβ) as its major component. In addition, the plaques consist of dystrophic neurites and periplaque

glial cells. NFTs are intracellular paired helical filaments consisting mainly of an abnormally phosphorylated form of the cytoskeletal protein tau. In addition to SPs and NFTs, also granulovascular degeneration, Hirano bodies and amyloid angiopathy are characteristic of AD histology. (Haberland 2007)

Aβ deposition follows a hierarchical sequence that can be divided into five phases. In the first phase, Aβ is seen only in the neocortex, with the temporal, occipital and frontal lobes showing the most consistent involvement. In the second phase, also the allocortex exhibits Aβ, including the entorhinal region, parts of the hippocampus and insular cortex. In phase three, Aβ progresses to diencephalic nuclei, putamen, caudate nucleus, substantia innominata and magnocellular nuclei of the basal forebrain, and several brainstem nuclei become involved in phase four. Finally, in phase five, Aβ depositions are seen in cerebellum and additional brainstem nuclei. Regions that become affected by Aβ pathology receive neuronal input from areas already exhibiting Aβ, but also regional susceptibility seems to determine when a given region becomes affected. (Thal et al. 2002)

NFTs have been shown to develop first in predisposed cortical induction sites, subsequently infiltrating other cortical regions in a consistent, predictable way. The lesions first emerge in the transentorhinal region and then appear in the entorhinal region. After that, NFTs develop in hippocampus, temporal and insular proneocortical areas and in some subcortical nuclei. NFTs are next seen in higher order neocortical multimodal association areas, spreading superolaterally, with inferior temporal areas showing earliest changes. Finally, NFTs are seen in primary motor and sensory as well as unimodal secondary cortices. The first stages of NFT infiltration are presymptomatic with symptoms of AD starting to emerge when NFTs invade hippocampus and multimodal association areas. (Braak et al. 1999)

In addition to histopathological changes, AD patients also display pathological changes in neurotransmitters. Basal forebrain cholinergic cell loss is consistently seen in AD, and impaired cortical cholinergic neurotransmission affects Aβ and tau pathology. There is also cell loss in locus coeruleus, and the level of norepinephrine becomes reduced in several cerebral regions. The chronic neuronal insult causes activation of extra-synaptic N-methyl-D-aspartate glutamate receptors, which in turn increases the intracellular calcium concentration. There is also evidence for an interaction between Aβ, glutamate receptors and oxidative stress in AD. (Mohandas et al. 2009)

Inflammation together with autotoxicity is also considered as being important in the pathogenesis of AD. The evidence for this comes from findings indicating that brain cells are capable of producing a spectrum of immune and inflammatory mediators, pentraxins – including C-reactive protein and amyloid P – are upregulated in AD, Aβ can activate complement, and a number of potent inflammatory cytokines become upregulated in the AD brain. (McGeer and McGeer 2001) Further evidence emerges from epidemiological studies suggesting that non-steroidal anti-inflammatory drugs are associated with reduced AD risk, even though clinical trials testing this hypothesis have failed to show a protective effect (McGeer et al. 2006).

Even though the histological aspects of AD are well-known, there has been controversy about whether it is the SPs or NFTs that represent the primary pathological process in AD. The well documented amyloid hypothesis originally proposed that the increased production or decreased clearance of Aβ peptides causes AD by leading to an accumulation of Aβ peptides, resulting in aggregation and formation of insoluble plaques (Hardy and Higgins 1992). Aβ is derived from the transmembrane amyloid precursor protein (APP) through cleavage by two proteases referred to as β- and γ-secretases. Three forms of Aβ are produced, comprising 38, 40 or 42 amino acid residues (Aβ-38, Aβ-40 and Aβ-42, respectively). Aβ-42 is important in AD because of its higher tendency to form oligomers and amyloid fibrils. (Walsh and Selkoe 2007)

Originally, SPs were presumed to trigger the cascade of deleterious changes resulting in AD. However, findings suggesting that SP accumulation correlates well with neither cognitive status (Giannakopoulos et al. 2003) nor brain atrophy (Josephs et al. 2008) imply that the plaques do not trigger the pathological events, but may be merely relatively inert sinks for misfolded proteins (Caughey and Lansbury 2003). Instead, it has been suggested that soluble Aβ oligomers are the primary factor in AD pathogenesis, even though the exact nature of these oligomers is still unknown (Klein 2002; Walsh and Selkoe 2007). The importance of oligomers is supported by the finding that the concentration of soluble Aβ correlates with synaptic loss (Lue et al. 1999). The level of soluble Aβ also correlates with the degree of neurodegeneration (McLean et al. 1999), and oligomeric Aβ can cause hippocampal and entorhinal but not cerebellar neuronal death (Klein et al. 2001).

As stated above, all research does not support amyloid pathology as the primary event in AD pathogenesis. Instead, it has been suggested that the formation of NFTs is the primary event. This hypothesis suggests that the normal function of tau in stabilizing microtubules is impaired in AD, and this causes neuronal loss of function. It is controversial whether the hyperphosphorylation of tau seen in AD is responsible for the dysfunction of tau or whether hyperphosphorylation occurs secondarily to the aggregation of tau into NFTs. (Mudher and Lovestone 2002) The hypothesis emphasizing the role of NFTs is supported by the intraneuronal accumulation of NFTs and by the good correlation of NFT accumulation with AD progression (Braak et al. 1999).

Much of the evidence suggesting that amyloid pathology is the primary event in AD comes from genetic studies. All known mutations causing the rare familial Alzheimer's disease (fAD) alter amyloid metabolism, and the neuropathology of fAD is similar to the more common sporadic AD. These mutations will be discussed in detail in chapter 2.3.3. In addition, mutations in the tau gene can cause FTD with hyperphosphorylated tau protein and filamentous pathology without amyloid pathology (Heutink 2000). Taken together, this suggests that the amyloid pathology lies upstream from the tau pathology in the pathogenesis of AD.

2.1.2.2 Clinical presentation

The clinical presentation of AD can be divided into four stages – early, mild, moderate and severe AD – which correlate with the progression of NFT pathology described above. During disease progression, episodic memory is constantly the most severely affected cognitive function, even though there are also rare non-amnestic subtypes. The onset is usually insidious and slowly progressing.

The first symptoms in early AD are difficulties in learning and pronounced forgetting, which represent symptoms of impaired episodic memory. There is difficulty in learning new subject matter and names, as well as in mastering foreign languages. Work becomes slower and more uncertain, and coping in new situations is difficult. However, patients are still able to live independently. Behavioral symptoms may appear, i.e. depression, anxiety and irritability. In the early stages, patients are symptom aware, but as the disease progresses this awareness attenuates, being replaced by an underestimation of symptoms. At this stage NFTs are usually seen in transentorhinal and entorhinal cortex as well as in hippocampus.

In mild AD, episodic memory and learning are impaired to a degree affecting daily activities and patients become dependent on memory aids. Remembering of individual events is still intact but the order of events becomes confused. In addition to the memory disorder, there are deficiencies in other cognitive domains as well, especially in verbal functions, initiation, attention, planning, and executive functions, and also difficulties in navigating foreign environments. Orientation starts to become impaired. Therefore, the criteria of dementia are met. Independence in more demanding daily activities is lost but with help patients can live alone. The most common behavioral symptoms in this stage are depression, irritation, apathy and emotional flatness, even though some patients express signs of paranoia. Pathologically, this stage correlates with NFTs appearing in the neocortex as well.

In moderate AD, symptom awareness is significantly impaired, and short term memory is severely affected. Almost all patients have impaired verbal functions as well, leading to further decreased fluency and problems in comprehension. Temporal and spatial orientation is severely impaired, and apraxia and agnosia emerge. Behavioral symptoms increase, as paranoia, depression, anxiety and apathy become increasingly common. Patients are no longer able to live independently, managing only short periods of time without supervision.

In severe AD, memory works only sporadically, talking and comprehension are minimal, orientation, concentration and perception are weak and apraxia severe. Nearly all patients express behavioral symptoms and need assistance in basic daily activities. Apractic gait, diminished balance reflexes and extrapyramidal symptoms all increase the risk of falls. Some patients become spastic and primary reflexes may re-emerge. Some patients also suffer myoclonus and epileptic seizures.

Early AD progresses slowly over several years. About every second patient with mild AD progresses to more severe AD during three years, and in five years, 75 % of

untreated patients with mild AD have been institutionalized. Time to death from symptom onset ranges from two to 16 years with an average of about ten years. (Erkinjuntti et al. 2010a)

2.1.2.3 Findings in structural imaging

Patients with advanced AD show diffuse cortical and cerebral atrophy in magnetic resonance imaging (MRI), but atrophy is first seen in the medial temporal lobe, including entorhinal cortex and hippocampus, and in posterior cingulate cortex. Subsequently atrophy is detected in the temporal, parietal and frontal cortices. Structural imaging is also useful in differentiating AD from other neurodegenerative conditions, and in excluding structural abnormalities that could cause cognitive deficits, including brain tumors, subdural hematomas and normal pressure hydrocephalus. (For review, see Frisoni et al. 2010)

As NFT pathology as well as atrophy in MRI are first seen in medial temporal structures, MRI changes in these structures have been studied in great detail. Visual and linear assessments have been used to evaluate different parameters, i.e. the width of the temporal horn, width of choroid fissure and height of the hippocampus, and many studies have used a visual rating scale ranging from absent to severe atrophy. The medial temporal lobe atrophy seen in MRI has been estimated to have a sensitivity of 85 % and specificity of 88 % in detecting clinically diagnosed mild to moderate AD. (Scheltens et al. 2002) The reduced hippocampal volume has also been shown to correlate with AD neuropathology post-mortem (Gosche et al. 2002). Hippocampal volume correlates inversely with AD severity, and hippocampal as well as entorhinal cortex atrophy effectively differentiates even patients with very mild AD from healthy controls (Dickerson et al. 2001; Jack et al. 1997). However, medial temporal lobe atrophy is not specific to AD, since it can be observed in DLB and other neurologic disorders as well (Dubois et al. 2007; Scheltens et al. 2002).

The concept that medial temporal lobe atrophy is the earliest and most sensitive marker of AD in MRI is supported by findings that this atrophy seems to differentiate those subjects with MCI who develop AD during follow-up from those who do not. Studies using quantitative analysis of hippocampal volume have shown that the volume is smaller in MCI patients developing AD in follow-up, with the degree of atrophy being especially pronounced in the lateral edge of the hippocampus (Apostolova et al. 2006; Jack et al. 1999). Similarly, increased medial temporal cortex atrophy in qualitative analysis is associated with an increased risk of converting from MCI to AD (DeCarli et al. 2007; Korf et al. 2004).

2.1.2.4 Cerebrospinal fluid markers

Three different cerebrospinal fluid (CSF) markers of AD have been introduced into clinical practice. Tau protein is located in neuronal axons, and the concentration of total tau in CSF is considered to reflect neurodegeneration in chronic neurodegenerative disorders. Total tau CSF concentrations are about three times higher in AD patients than in controls, and increased tau has been shown to have a specificity of 90 % and

sensitivity of 81 % for detecting AD. The Aβ-42 CSF concentration is decreased in AD, and reduced Aβ-42 has a specificity of 90 % and sensitivity of 86 % for AD. The decrease in the Aβ-42 concentration possibly indicates deposition of this peptide into SPs. CSF concentration of phosphorylated tau (p-tau) is increased in AD, which is considered to reflect the phosphorylation state of tau and, hence, the formation of NFTs. The specificity and sensitivity of elevated p-tau for AD is 92 % and 80 %, respectively. (Blennow and Hampel 2003) A recent meta-analysis concluded that CSF tau concentrations in patients with DLB, FTD or VaD are moderately elevated as compared to controls, whereas concentrations of p-tau seem to be more control-like, thus differing more significantly from AD patients (van Harten et al. 2011).

2.1.2.5 Diagnostic criteria

The golden standard of AD diagnosis is still a neuropathological post-mortem examination. The most often applied consensus recommendations for the neuropathological diagnosis of AD are those by the National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease (1997) (NIA-RI 1997), which assign either high, intermediate or low likelihood of AD based on neuropathological findings from multiple neocortical areas, hippocampal formations, substantia nigra and locus coeruleus. The NIA-RI 1997 recommend using the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) (Mirra et al. 1991) and Braak criteria (Braak and Braak 1991) to assess SP and NFT burden, respectively.

Since at present there is no definitive diagnostic biomarker for sporadic AD, the clinical diagnosis cannot be definite but only probabilistic. Most scientific work published during the last decade has used the National Institute of Neurological Disorders and Stroke – Alzheimer's Disease and Related Disorders (NINCDS-ADRDA) criteria from 1984 (McKhann et al. 1984). The criteria are fulfilled in a two-step process in which a dementia disorder is diagnosed first, and only after that are the criteria for features more specific of AD applied. The criteria for clinically probable AD are that dementia has been established by clinical and neuropsychological examination, cognitive impairment must be present in at least two cognitive domains, and the impairment of memory and cognition must be progressive. The onset of symptoms must occur between 45 and 90 years of age, and usually occurs after the age of 65. The accuracy of the NINCDS-ADRDA criteria has been addressed in multiple studies using neuropathologic diagnosis as the reference. The sensitivity of probable AD diagnosis has been found to range from 49 to 100 % in individual studies with an average of 81 %, with specificities varying from 47 up to 100 %, averaging 70 %. (Knopman et al. 2001)

The clinical phenotype of AD is now better understood than when the NINCDS-ADRDA criteria were proposed. The criteria are also based on the assumption that AD is a clinical-pathological entity with a close correlation between pathology and clinical presentation. This has however been shown to be incorrect, and pathological changes of AD begin to accumulate approximately 10-15 years before clinical manifestation (Jack et al. 2011; Perrin et al. 2009). The criteria do not include any biomarkers with the

ability to show the presence of AD pathogenesis *in vivo* prior to death. To address these and other challenges in AD diagnosis, two new diagnostic criteria have been proposed (Dubois et al. 2007; Jack et al. 2011; McKhann et al. 2011).

The National Institute on Aging-Alzheimer's Association workgroups proposed new diagnostic criteria for AD in 2011 (Jack et al. 2011; McKhann et al. 2011). The criteria still use a two-step approach. Dementia is diagnosed when cognitive or behavioral symptoms interfere with the ability to function at work or usual activities, represent a decline from previous levels, and include deterioration in at least two cognitive domains. In addition, the diagnosis of probable AD dementia requires that the onset of symptoms is insidious, that there is a clear-cut history of worsening cognition, and that the initial and most prominent cognitive deficit is either amnestic or in accordance with the non-amnestic presentations of AD. The diagnosis of possible AD dementia is suggested when symptoms have a sudden onset or there is not enough evidence of progressive decline. Additionally, a diagnosis of possible AD is made in cases with additional conditions that could have a substantial effect on cognition, e.g. concomitant cerebrovascular disease. The role of biomarkers is still limited, and they are divided into markers of Aβ pathology and downstream neuronal injury. The former parameters include low CSF Aβ-42 and positive PET amyloid imaging, whereas the latter include decreased glucose metabolic rate in PET in temporoparietal cortex, increased CSF tau or p-tau, and disproportionate atrophy of medial, basal and lateral temporal lobe and medial parietal cortex in MRI. In patients with a non-AD phenotype, the diagnosis of possible AD dementia with evidence of AD pathophysiology can be made only if both categories of biomarkers are positive.

Dubois et al. (2007) suggested diagnostic criteria that would emphasize the role of biomarkers. According to their criteria, the diagnosis of probable AD requires the report of a gradual and progressive change in memory function with objective evidence of significantly impaired episodic memory on testing. Notably, the criteria do not require a dementia level cognitive impairment, as the memory impairment can be isolated. Additionally, to fulfill the criteria of AD, there must be evidence of medial temporal lobe atrophy in MRI, abnormal CSF markers, a specific pattern on PET imaging (e.g. reduced glucose metabolism in bilateral temporal parietal regions), or a proven AD autosomal dominant mutation in the immediate family.

It must be noted that neither of the proposed new criteria have been extensively validated, and large follow-up studies will be required to ascertain their sensitivity and specificity against a neuropathological diagnosis.

2.1.2.6 Treatment

No disease modifying treatment is available for AD, and the currently available medications provide only modest symptomatic relief. Cholinesterase inhibitors prevent the breakdown of acetylcholine by inhibiting the acetylcholinesterase enzyme. The three cholinesterase inhibitors available – donepezil, galantamine and rivastigmine – have slightly different pharmacological properties but share the same pharmacodynamic

mechanism. They are fairly well tolerated and all show similar efficacy in improving cognitive function, global clinical state and activities of daily living, but the treatment effects are not impressive. Even though most of the evidence comes from patients with moderate AD, there is no evidence indicating that they would be less effective in mild or severe AD. (Birks 2006)

In addition to the cholinesterase inhibitors, also memantine, a low affinity N-methyl-D-aspartate type glutamate receptor antagonist, is indicated in moderate to severe AD. Enhancement of the excitatory effect of glutamate is associated with AD pathogenesis and damage due to ischemic stroke, and memantine may protect from this neurotoxicity without interfering with the physiological effects of glutamate. Memantine is effective in patients with moderate to severe AD and improves or preserves cognition, mood, behavior, and the ability to perform daily activities. Memantine might also be effective in mild AD. Interestingly, memantine has a positive effect on cognition also in mild to moderate VaD, but this effect is not reflected in the clinical impression. (McShane et al. 2006)

2.1.3 Vascular cognitive impairment

Cerebrovascular disease can cause a decline in cognitive function extending beyond traditional multi-infarct dementia, and furthermore cognitive impairment associated with cerebrovascular disease often does not fulfill the criteria for dementia. Therefore, instead of VaD, the term vascular cognitive impairment (VCI) has been proposed. Instead of representing a single clinical entity, VCI can be divided in multiple separate disorders including multi-infarct dementia, subcortical ischaemic vascular dementia (SIVD) and cognitive impairment caused by a strategically located infarction.

The most common form of VCI is SIVD with a clinical presentation characterized by psychomotor slowness due to impaired executive functions as well as changes in speech, affect and mood. Deterioration in memory is less severe than in AD, and manifests mainly as forgetfulness and difficulties in spontaneous recall. Physical signs include signs of mild upper motor neuron dysfunction (arm drift, reflex asymmetry or central facial weakness), small-step gait, unsteadiness, extrapyramidal signs, pseudobulbar palsy and urinary symptoms. The personality and mood disorders include apathy, irritability and depression. White-matter lesions and lacunar infarcts are seen in structural imaging. The white-matter lesions consist of extensive periventricular lesions and deep lesions affecting especially the anterior limb of the internal capsule, anterior corona radiata and anterior centrum semiovale. Lacunar infarcts are often seen in the caudate, globus pallidus, thalamus, internal capsule, corona radiata, and frontal white matter. The lesions are distributed such that they can disrupt the prefrontal subcortical circuits, which is consistent with the clinical presentation. (Erkinjuntti et al. 2010b; Roman et al. 2002)

The less common multi-infarct dementia is characterized by multiple cortical or cortico-subcortical infarcts. Disease onset is often abrupt with a step-wise progression. Clinical presentation is characterized by uneven performance in different cognitive domains, and common early symptoms include often mild memory impairment, deterioration in

executive functions and cortical symptoms (e.g. aphasia, apraxia or agnosia). Visuospatial and constructional difficulties can also be encountered. The findings in clinical examination depend on the location of the infarcts and can include visual field defects, hemiparesis, gait disorder or central facial weakness. (Erkinjuntti et al. 2010b)

Small vessel disease is now considered as the most important factor involved in VCI pathogenesis, even though large vessel occlusions causing extensive cortical infarcts do cause multi-infarct dementia. Complete or incomplete infarctions are associated with small vessel disease. Incomplete infarctions may evoke white-matter lesions and are associated with more chronic, diffuse and less severe ischemia leading to selective tissue damage. Small vessel disease also causes lacunar infarcts and cortical brain atrophy. (O'Brien et al. 2003)

Multiple clinical criteria have been suggested for VaD, but the National Institute of Neurological Disorders and Stroke International Workshop and Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) criteria are most often applied for research purposes (Roman et al. 1993). The criteria require both dementia and cerebrovascular disease, as well as a relationship between the two. Cerebrovascular disease is considered to include focal signs on neurologic examination, and evidence of relevant cerebrovascular disease in brain imaging with multiple large vessel infarcts, or a single strategically placed infarct, multiple basal ganglia and white matter lacunas, or extensive periventricular white matter lesions. The relationship between dementia and cerebrovascular disease is inferred by the onset of dementia within 3 months following a stroke, an abrupt deterioration in cognitive function or a fluctuating, stepwise progression of cognitive deficits.

2.2 Twin studies

2.2.1 Principles and applications of twin research

The different genetic characteristics of MZ and DZ twins form the basis of twin research. MZ co-twins have identical genomes at the DNA sequence level, but their functional genome may differ due to epigenetic changes and somatic mutations. In contrast, DZ co-twins share, on average, half of their segregating genes. Both MZ and DZ co-twins are considered to share common environmental factors to the same degree. Concordance is defined as the occurrence of the same trait or disease in both members of a twin pair, and thus any trait or disease with a genetic component will have higher concordance in MZ than in DZ twins. Heritability is the proportion of variation in a trait that can be attributed to additive genetic effects, and classical twin studies allow for the estimation of both heritability and the proportion of the variance due to a shared environment using correlations between co-twins.

More advanced analyses allow testing the hypothesis of whether different genes influence a trait or disease in opposite sexes and, more importantly, testing for possible genotype × environment interactions through inclusion of environmental factors on which the twins can be stratified. (Boomsma et al. 2002) Testing for possible

environmental and genetic correlations between traits is achieved through multivariate analyses. These permit an analysis of whether a correlation between two or more traits is due to common genetic effects, environmental factors affecting them both, or a causal relationship between them. Using longitudinal data also allows one to test whether different genetic factors are effective at different time points or ages. (Kaprio and Silventoinen 2011) Another approach is to study co-twins who are discordant for either a disease or exposure. Differences between MZ co-twins are caused by environmental differences between them, which makes this co-twin control design an extremely useful tool for investigating causes or consequences of a disease. However, the possible environmental differences include very different effects affecting over a long time span. It is even possible that the effective genotypes of MZ co-twins begin to diverge over time due to epigenetic and environmental effects modifying gene expression. (Kaprio and Silventoinen 2011)

Due to the unique characteristics of twins, multiple national twin cohorts have been established. The number of individuals in these cohorts is impressive, for example cohorts of the GenomEUtwin project contain a total of about 0.8 million twins. Apart from the twin structure, the cohorts are usually unselected and population based. Therefore, they represent the full Gaussian variety of different traits and are free of ascertainment bias. Members of the longitudinal twin cohorts have participated in the studies mainly by answering questionnaires or interviews, and twins also provide access to data from core family members. (Peltonen and GenomEUtwin 2003) Even though twins have some pregnancy and infancy characteristics that differentiate them from singletons, they are highly representative of the general population with respect to nearly all traits after pregnancy and early childhood (Kaprio and Silventoinen 2011).

2.2.2 The Finnish Twin Cohort and specific advantages of twin studies in Finland

The Finnish Twin Cohort was established in 1974. Ascertainment of twins and their families has been done from the Central Population Register in 1974, 1987 and 1995. The older part consists of same-sex twin pairs born before 1958 with both co-twins alive in 1975. Postal questionnaires have been carried out in 1975, 1981 and 1990 with good response rates. The twins have been followed for mortality and morbidity through linkage to multiple national registries, including the Population Register Centre, Cancer Registry, Hospital Discharge Registry and the national registry for fully reimbursable medications. Additionally, numerous sub-sample studies have been performed since the 1990s, and the current study is based on one of these sub-samples. (Kaprio and Koskenvuo 2002; Peltonen and GenomEUtwin 2003)

Finland and its population have a number of features making them well suitable for twin cohort studies. Firstly, Finland has been considered to be genetically rather isolated, which confers a higher degree of genetic homogeneity. Additionally, environmental homogeneity during fetal life and childhood minimizes problems associated with analyses of complex diseases. Finland also has unique genealogical data in church records containing information on births, deaths, marriages and movements for the

majority of the population dating back to 1640. Finally, Finland has a uniform and well-developed health care system with computerized records producing high quality registers on mortality and morbidity. (Kaprio and Koskenvuo 2002)

2.3 Genetics of Alzheimer's disease

2.3.1 Evidence of genetic factors in Alzheimer's disease

It is well established that the more common sporadic form of AD is a multifactorial disorder, implying that both genetic/familial and environmental factors contribute to its development. In the Multi-Institutional Research in Alzheimer's Genetic Epidemiology (MIRAGE) study, 12 971 first-degree relatives of 1 694 AD patients were examined. The first-degree relatives had an estimated lifetime AD risk of 39 %, which was estimated to be approximately twice the estimated cumulative incidence of AD in the general population. However, the risk was significantly less than 50 %, implying that autosomal dominant or co-dominant inheritance is unlikely to explain the aggregation of AD in families. (Lautenschlager et al. 1996) Further analysis of the MIRAGE study concluded that the risk attributable to familial aggregation is similar in African American and white families (Green et al. 2002).

Further evidence for the importance of genetic factors in sporadic AD has emerged from twin studies. Twins in the older Finnish Twin Cohort were cross-checked with the hospital discharge registry to find subjects with dementia, and dementia diagnoses were confirmed through a review of medical records. Based on data from 268 same-sex twin pairs, it was estimated that the pairwise concordance of AD was 50 % in MZ males, 11.4 % in MZ females and 6.7 % in DZ females. The corresponding probandwise concordance rates were 67 %, 21 % and 12.5 %. There were no concordant DZ male pairs and only eight MZ male pairs were studied. (Raiha et al. 1996) In a similar study, cognitively impaired subjects of the Norwegian Twin Registry residing in long-term institutions were identified, and 72 twin pairs were recruited for further clinical diagnostic work-up. Pairwise and probandwise concordances of AD were 78 % and 83 % in MZ twins, respectively, and 39 % and 46 % in DZ twins. By using tetrachoric correlations and adjusting for sampling, the heritability was estimated at 0.55-0.61. (Bergem et al. 1997) In both of these studies subjects with less severe AD were probably overlooked, which could introduce some bias to the results.

The largest and most comprehensive study to date on AD concordance and heritability is based on the Swedish Twin Registry. In 1998-2001, all subjects in the registry born in 1935 or earlier were identified and asked to participate in a telephone interview assessing their cognitive function. Subjects screened positive for cognitive impairment were then clinically assessed for dementia. 4 225 complete twin pairs including same-sex as well as opposite-sex pairs were studied. Probandwise concordance for AD was 45 % in MZ men, 61 % in MZ women, 19 % in DZ men, 41 % in DZ women and 21 % in opposite-sex twins. The higher concordance rates in women were attributed to their greater longevity. There was no evidence that the heritability of AD differs in men and women or that different genetic effects operate in the genders. To prevent inflated shared

environmental effects, also age was controlled for in the heritability analyses, and heritability was estimated at 0.58 in a full model (95 % confidence interval [CI] 0.19-0.87). Additionally, differences in age of onset were greater in concordant DZ than MZ twins, suggesting that genes also modify the age of onset of AD. (Gatz et al. 2006)

2.3.2 Role of Apolipoprotein E ε4 in sporadic AD

Apolipoprotein E (ApoE) ε4 allele is the most widely studied individual genetic risk factor for AD. Apolipoprotein E is a glycoprotein existing in three isoforms, namely ApoE2, ApoE3 and ApoE4 coded by the alleles ε2, ε3 and ε4, respectively. The alleles are determined by two single-nucleotide polymorphisms, and estimated prevalences of the alleles are 6 %, 78 % and 15%, respectively, but the prevalences vary between different latitudes (Eisenberg et al. 2010). The first studies suggesting that ApoE ε4 is associated with AD were published in 1993. Strittmatter et al. (1993) compared 30 subjects from different AD families to 91 unrelated controls and found the ε4 allele to be overrepresented in AD patients. In a subsequent study, Saunders et al. (1993) confirmed that the ε4 allele was overrepresented also in sporadic AD patients. Shortly thereafter the protective effect of the ε2 allele was discovered (Corder et al. 1994).

Since the original findings relating ApoE genotype to AD, multiple studies have been published on the association. In 1997 raw data of 40 research teams including 5 930 AD patients and 8 607 controls were meta-analyzed. In Caucasians, the risk of AD was increased in subjects with ε4/ε4, ε3/ε4 and ε2/ε4 genotypes compared to subjects with ε3/ε3 genotype (odds ratios [OR] 12.5-14.9, 2.7-3.2 and 1.2-2.6, respectively). Among African Americans, the risks associated with ε3/ε4 or ε2/ε4 genotypes were statistically non-significant, and the risk associated with ε4/ε4 genotype clearly lower than in Caucasians (OR 5.7). The ORs were also lower among Hispanics than among Caucasians, with a significant risk associated with ε3/ε4 (OR 2.2) but not with ε4/ε4 genotype. In contrast, among Japanese subjects, the risk associated with ε4 allele was stronger than among Caucasians (OR 5.6 for ε3/ε4 genotype, and OR 33.1 for ε4/ε4 genotype). In addition, a protective effect of the ε2 allele was observed and the OR associated with ε2/ε3 genotype was about 0.6 in all populations. These results suggest a dose-dependent increase in risk of AD associated with the ε4 allele, but the risk seems to vary between different ethnic populations. (Farrer et al. 1997)

The ε4 allele seems to have an age-dependent effect on AD risk. It is most prevalent in patients with age of onset between 60-69 years and less common in subjects with both younger and older ages of onset, suggesting that the ε4 allele has a maximum impact on subjects with age of onset between 60 and 70 years (Davidson et al. 2007). These findings are in line with the findings of the meta-analysis described above.

Lipoproteins mediate the transport and clearance of lipids. In the brain, ApoE is primarily expressed in astrocytes, microglia and oligodendrocytes, and contributes to the transport of cholesterol and phospholipids to sites of neuronal membrane regeneration and remyelination. Multiple mechanisms have been proposed to account for the association between ApoE4 and AD. ApoE4 may be more vulnerable to aberrant

degradation than other isoforms. ApoE4 is also characterized by its greater ability form C-terminal-truncated fragments which stimulate tau hyperphosphorylation and the formation of NFTs. In addition, Aβ clearance is less efficient in the brains of ApoE ε4 carriers, and ischemic brain insults and white matter lesions tend to be greater in ε4 carriers. (Schipper 2011) However, the final mechanism has not been unambiguously clarified.

2.3.3 Familial Alzheimer's disease

In contrast to the multifactorial sporadic AD, fAD is inherited dominantly in the autosome. fAD is extremely rare compared to the multifactorial sporadic form, and it is characterized by an earlier age of onset before the age of 65; a proportion of subjects with fAD even develop dementia before the age of 50. Thus far, mutations in three genes have been identified to cause fAD: APP in chromosome 21, presenilin-1 (PSEN-1) in chromosome 14 and presenilin-2 (PSEN-2) in chromosome 1. It has been estimated that fAD caused by mutations in these genes accounts only for about 0.5 % of all AD, and that 14 %, 81 % and 5 % of fAD cases are caused by mutations in APP, PSEN-1 and PSEN-2, respectively (Ertekin-Taner 2007). As of September 2011, 32 mutations causing fAD have been identified in 89 families in APP, 185 mutations in 405 families in PSEN-1, and 13 mutations in 22 families in PSEN-2 according to the Alzheimer Disease and Frontotemporal Mutation Database (http://www.molgen.ua.ac.be/ADMutations).

The genes associated with fAD are all important in the formation of Aβ and hence support the amyloid hypothesis. Mutations in APP causative of fAD reside close to the cleavage sites of the secretases and lead to increased formation of Aβ-42 (Hardy 1997). Furthermore, the mutations in PSEN-1 and PSEN-2 lead to increased formation of Aβ-42, and it has been shown that both presenilins are required for γ-secretase activity, suggesting that the presenilins are part of the γ-secretase complex (Hardy 1997; Zhang et al. 2000).

2.4 Positron emission tomography

2.4.1 Principles and physical background of PET

PET is an analytical imaging technology utilizing tracers labeled with positron emitting radioisotopes. It enables functional imaging and measurement of biochemical processes *in vivo*. In comparison to other isotope imaging, PET enables quantitative analysis and has superior resolution and sensitivity. The most commonly used isotopes are fluorine-18, carbon-11, oxygen-15 and nitrogen-13; fluorine is used as a hydrogen substitute since there is no positron emitting isotope of hydrogen. (Knuuti et al. 2000) PET tracers only track a small number of steps in the studied biochemical processes so that kinetic analysis can be used to estimate the concentration of reactants and products over time, allowing for the calculation of reaction rates. In practice, the tracer is most often injected intravenously and the PET scan provides temporal data of the tracer tissue concentration. As this is combined with tracer plasma concentration over time representing tracer

delivery to the tissue, an image of the rate of the assessed process can be generated with the use of compartmental models. (Phelps 2000)

The radioisotopes used in PET are beta-positive implying that as they undergo positron emission decay, their nuclei emit positrons, or antiparticles of electrons with an opposite charge. As a positron travels through tissue, it loses its kinetic energy, and after travelling for about one millimeter it stays in the proximity of an electron long enough for annihilation to happen. In annihilation, two 511 keV gamma photons travelling in approximately opposite directions are formed. PET scanners have multiple detectors on a ring that detect these gamma photons. When two of the detectors are impacted by a photon within a certain time frame, it can be inferred that these photons coincide and, hence, must have originated from the same annihilation. This places the annihilation in a space joining the two detectors, or their line of response (LOR). As photons travel through tissue they are subject to attenuation, which can be corrected for by calculating a linear attenuation coefficient for each LOR. Additionally, one can correct for scatter and random coincidence. After these corrections, the number of annihilations assigned to each LOR joining two detectors is proportional to the integral of activity along that LOR. Parallel sets of such LORs are called projections, and these projections are then used to reconstruct the final PET images. (Badawi 1999)

2.4.2 PET applications in Alzheimer's disease and dementia

2.4.2.1 General considerations

PET has been used to examine cerebral blood flow, glucose metabolism and neurotransmitters in AD and dementia patients. Most recently, the development of new tracers has made it possible to examine the primary pathological events of AD *in vivo*. (Herholz et al. 2007) The current and possible future applications of PET in dementia research and clinical practice involve its use as a diagnostic tool, in treatment follow-up, and in testing the efficiency of new therapeutic agents. The review presented here is limited to imaging of cerebral glucose metabolism and amyloid deposition.

2.4.2.2 Imaging of regional glucose metabolic rate

The structure of 2-deoxy-2-[^{18}F]fluoro-D-glucose (FDG) is identical to that of glucose except for the 2-position of the molecule, which has a fluorine-18 atom attached to it instead of a hydroxyl group. It is the most commonly used tracer in PET because it reflects glucose metabolism. After intravenous injection, FDG is efficiently extracted by cells, and its transport into cells is similar to that of glucose. In cells, like glucose, FDG is first phosphorylated by hexokinase to 2-deoxy-2-[^{18}F]fluoro-D-glucose-6-phosphate. In contrast to phosphorylated glucose, 2-deoxy-2-[^{18}F]fluoro-D-glucose-6-phosphate is not metabolized further as it is not a substrate for glucose-6-phosphate-isomerase and neither is it dephosphorylated, because the enzyme responsible for this process is not present in significant amounts in the studied tissues. Therefore, FDG is 'metabolically trapped', permitting a specific evaluation of tissue glucose utilization. (Beuthien-Baumann et al. 2000)

A compartmental model is used to evaluate the glucose metabolic rate (GMR). Originally, a model was proposed for measuring GMR in rat brain using [^{14}C]deoxyglucose (Sokoloff et al. 1977), but this has been modified for use in PET with FDG (Phelps et al. 1979; Reivich et al. 1979). The model assumes three compartments: plasma, dephosphorylated FDG in the tissue and phosphorylated FDG in the tissue with dephosphorylation being presumed to be minimal. The metabolism of FDG and the three compartment model are illustrated in **Figure 1**. Later a graphical method was developed to directly estimate a combination term of the original model's constants, making assessment of GMR both fast and robust (Patlak and Blasberg 1985).
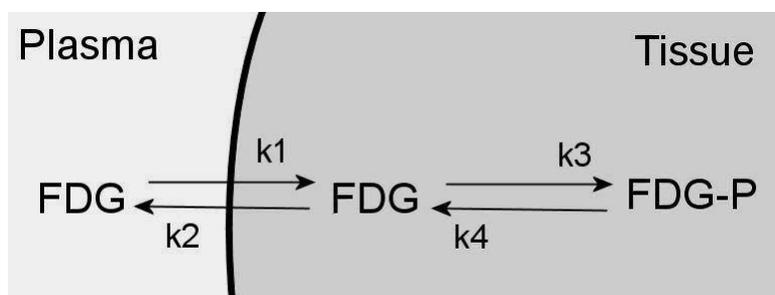

Figure 1. Illustration of FDG metabolism. FDG-P is phosphorylated FDG. In the three compartment model, k4 is assumed to be minimal.

The first FDG-PET studies on AD patients were published in the 1980s. They examined a limited number of AD patients, but revealed that GMR was globally reduced in AD patients compared to controls, most significantly in temporoparietal and frontal cortices with relative sparing of primary motor and sensory cortices (Benson et al. 1983; Friedland et al. 1983; McGeer et al. 1986) (**Figure 2**). They also provided preliminary evidence that the findings were not only a result of cerebral atrophy in AD patients (McGeer et al. 1986). During the following years, these findings have been verified in multiple studies, highlighting that GMR reductions are most severe in temporo-parietal association cortex with the angular gyrus cortex at the center of the reductions (Herholz 2003), and voxel-level analyses have indicated that the earliest reductions can be detected in posterior cingulate cortex (Minoshima et al. 1997). With the progression of AD, the reductions become increasingly extensive and severe in temporal and parietal cortex, and reductions in frontal cortex become more evident (Herholz et al. 2007). The pattern of reduced GMR in early AD differs from that of normal aging, in which GMR is reduced most significantly in frontal cortex, anterior temporal cortex, parietotemporal junction cortex and anterior cingulate cortex. GMR is also reduced in the vicinity of the ventricles due to age-related enlargements of CSF spaces. However, age-related reduction of GMR is also seen in posterior cingulate cortex. (Petit-Taboue et al. 1998; Zuendorf et al. 2003)

Studies on MCI subjects have shown that FDG-PET can be used to detect the very early changes in brain metabolism associated with AD pathology, even though there has been some inconsistency in the actual topography of changes in these subjects. Chetelat et al.

(2003) compared MCI subjects with healthy controls, and those MCI patients who progressed to AD during an 18-month follow-up exhibited reduced regional glucose metabolic rate (rGMR) in right posterior cingulate gyrus and right superior temporal gyrus. Similarly, Drzezga et al. (2003) showed that MCI subjects progressing to AD during a one-year follow-up displayed reduced rGMR at baseline in hippocampal and parahippocampal, inferior prefrontal, anterior insular, inferior parietal, and posterior cingulate cortex. In addition to showing differences in brain metabolism between controls and MCI subjects converting to AD, FDG-PET has also been shown to be able to differentiate MCI patients who develop AD from those who do not. The brain regions proposed as being associated with probable progression to AD in these studies include temporoparietal cortex (Arnaiz et al. 2001), right temporal gyrus and precuneus (Drzezga et al. 2003), posterior cingulate cortex (Chetelat et al. 2003; Drzezga et al. 2003), and right inferior parietal cortex (Mosconi et al. 2004).
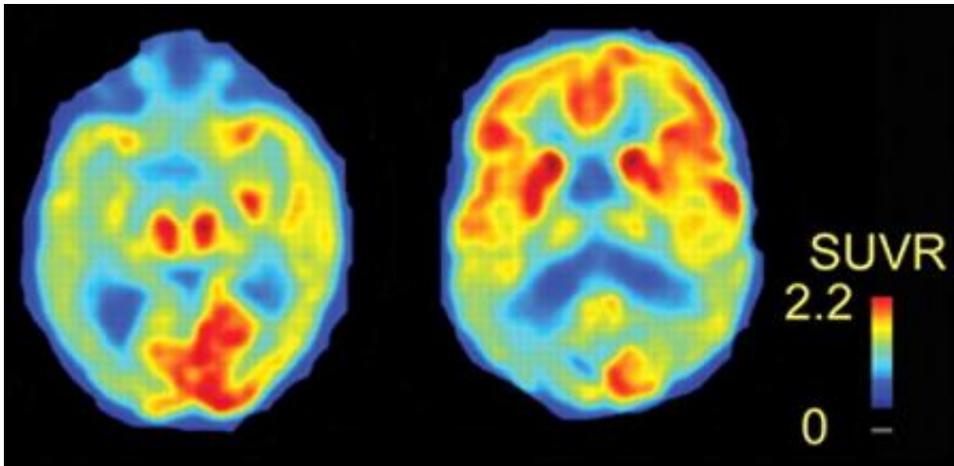
Figure 2. Typical pattern of GMR in AD with reduced metabolic rate most notably in temporal and parietal cortices. Image modified from Rabinovici et al. 2011.

The encouraging findings that changes in brain metabolism can be detected even in early AD raises the question of whether FDG-PET can be used as a diagnostic tool in clinical practice. In 2004, a meta-analysis surveyed applicable studies between 1989 and 2003, and the final analysis of nine studies indicated that both the sensitivity and specificity of FDG-PET in differentiating AD subjects from healthy controls was 86 %. However, there was a high degree of heterogeneity in these estimates between the individual studies. FDG-PET also seemed to have less optimal specificity in differentiating AD subjects from patients with other dementias. The included studies suffered from serious limitations, including an imperfect reference standard, presence of verification bias, and inconsistencies in the choice of disease spectrum. (Patwardhan et al. 2004) In a large multicenter study including 395 AD patients and 110 controls, a completely automated voxel-level analysis of FDG-PET revealed a sensitivity of 93 % and specificity of 93 % in differentiating AD patients from cognitively intact subjects. Even when including

only subjects with very mild dementia, sensitivity and specificity were still 83 % and 82 %, respectively. (Herholz et al. 2002) In addition to the clinical diagnosis of AD, the diagnostic accuracy of FDG-PET has also been compared to neuropathological diagnosis, partly because of the inaccuracy associated with a clinical diagnosis. A study of 138 subjects with FDG-PET scans and subsequent neuropathological confirmation of diagnosis on average three years later found that FDG-PET had a sensitivity of 94 % and a specificity of 73 % for detecting AD. Even in subjects with questionable or mild dementia at the time of the PET scan, sensitivity was 95 % and specificity 71 %. (Silverman et al. 2001)

Cognitively intact ApoE ε4 carriers represent individuals with a genetically mediated increased risk of AD, and their cerebral GMR has been studied in detail. A study of 50-84 year-old subjects free of dementia showed that ApoE ε4 carriers had reduced rGMR in inferior parietal, lateral temporal and posterior cingulate cortices compared to non-carriers. rGMR also predicted a decline in memory performance during a two-year follow-up. (Small et al. 2000) A similar study including late-middle-aged subjects found reduced rGMR in ApoE ε4 carriers as compared to non-carriers in posterior cingulate, precuneus, parietotemporal and frontal regions, and the ApoE ε4 gene dose correlated inversely with rGMR in these areas but not elsewhere in the brain. However, there was overlap between rGMR between ε4carriers and non-carriers. (Reiman et al. 2005) These findings are unlikely to represent a very early phase of AD, as even 20-39 year-old ApoE ε4 heterozygotes have lower rGMR in posterior cingulate, parietal, temporal and prefrontal cortices than non-carriers (Reiman et al. 2004). Additionally, ApoE ε4 seems to be associated with a more pronounced reduction in rGMR in AD subjects, even when controlling for age, education and dementia severity (Drzezga et al. 2005).

Other FDG-PET studies have concentrated on subjects who have an increased familial risk of AD distinct from the ApoE genotype. It has been shown that elderly cognitively intact subjects with a maternal family history of AD have reduced rGMR in a pattern typically observed in AD when compared to subjects with a paternal or no family history (Mosconi et al. 2007). Additionally, MZ twin pairs discordant for AD have been studied. Luxenberg et al. (1987) studied one such twin pair, and the demented co-twin was found to have reduced rGMR in frontal and parietal lobes, whereas no reductions were seen in the non-demented co-twin. Kumar et al. (1991) examined three discordant MZ twin pairs and found that the rGMR abnormality was limited to the demented co-twins with no abnormalities being seen in the non-demented co-twins. Small et al. (1993) reported rGMR to be normal in the non-demented co-twin in a discordant MZ twin pair, even though the non-demented co-twin had evidence of atrophy in MRI.

Prior to the current study, the largest FDG-PET study on twin pairs discordant for AD studied seven female MZ twin pairs discordant for AD using traditional region of interest (ROI) analysis. The cognitively intact co-twins showed reduced rGMR in temporal and parietal cortex. (Järvenpää et al. 2003) However, since no DZ twin pairs were included, it was impossible to differentiate between the effects of genetic and

shared environmental or familial factors, and due to methodological limitations, only a small part of brain space could be assessed for possible differences in rGMR.

Other causes of dementia involve changes in cerebral glucose metabolism distinct from AD. A voxel-level FDG-PET study revealed that subjects with SIVD had lower metabolism in basal ganglia, thalamus, cerebellum, sensory motor, dorsal premotor and auditory cortices, middle temporal gyrus, visual cortices, and anterior cingulate gyrus compared to AD patients (Kerrouche et al. 2006). A study examining AD and DLB patients indicated that rGMR values in DLB patients do not differ from those of AD patients in regions typically affected in AD – including posterior cingulate, lateral temporal and superior parietal cortex – but DLB patients show significantly lower rGMR in the visual primary and association areas as compared to AD patients (Gilman et al. 2005). Finally, FTD causes a reduction in rGMR mainly in the anterior parts of the brain consisting of frontal lobes, anterior temporal cortex and anterior cinculate cortex. FDG-PET combined with clinical assessment has superior accuracy compared to a clinical assessment alone in differentiating between AD and FTD patients. (Foster et al. 2007)

2.4.2.3 Cerebral amyloid imaging

Thioflavin T is a commonly used amyloid binding dye in *in vitro* studies, and its uncharged derivate $[^{11}C]$6-OH-benzothiazole-1, or $[^{11}C]$Pittsburgh Compound B (PIB) has been shown to be a suitable amyloid binding PET tracer (Bacskai et al. 2003; Lopresti et al. 2005; Mathis et al. 2003). Compared to controls, AD patients have greater standardized PIB uptake in frontal, parietal, temporal and occipital cortex as well as in striatum, but not in pons, subcortical white matter or cerebellar cortex. This topography is generally consistent with the amyloid deposition observed post-mortem in AD patients (Thal et al. 2002). rGMR and PIB retention also correlate to some extent, although only in parietal cortex has the correlation been shown to be statistically significant. (Klunk et al. 2004) Voxel-level studies have revealed the increased uptake to be most prominent in frontal cortex but significant also in parietal, lateral temporal and posterior cingulate cortex. In striatum, there is a considerable overlap with healthy controls. (Kemppainen et al. 2006) Subjects with amnestic MCI have been shown to exhibit increased PIB uptake in a similar pattern to AD patients but with a considerable overlap with healthy controls (Kemppainen et al. 2007). PIB-PET is also able to differentiate subjects with amnestic MCI progressing to AD from stable MCI patients (Okello et al. 2009). These findings suggest that PIB-PET is able to detect changes in the amyloid burden even in very early AD.

One limitation of PIB is the tendency of a proportion of cognitively preserved individuals to show increased PIB uptake (Aizenstein et al. 2008; Rowe et al. 2007). PIB uptake seems to increase with advancing age, and the ApoE ε4 allele increases PIB uptake in a dose-dependent manner in cognitively intact individuals (Morris et al. 2010). Additionally, PIB uptake is higher and more extensive in AD patients with at least one ε4 allele compared to patients without an ε4 allele (Drzezga et al. 2009).

Interestingly, it seems that PIB uptake in AD patients does not significantly increase in follow-up (Scheinin et al. 2009). A follow-up study of 20 patients with mild AD utilizing both FDG and PIB-PET showed that reduced GMR expanded more than PIB uptake during follow-up. Additionally, the progression of reduced GMR seemed to be limited to those areas already affected by amyloid deposition, suggesting that the topography of reduced GMR follows that of amyloid deposition with a temporal delay. (Forster et al. 2011)

A recent study including nine MZ and eight DZ Finnish twin pairs discordant for cognitive impairment showed that the cognitively preserved MZ co-twins had increased PIB uptake in lateral temporal cortex, inferior parietal cortex, posterior cingulate and putamen compared to healthy controls. In contrast, no differences were observed between the cognitively preserved DZ co-twins and controls. (Scheinin et al. 2011)

Studies comparing FDG-PET and PIB-PET in differentiating AD patients from healthy controls have reported inconsistent results. A study comparing visual and quantitative analysis of FDG-PET and PIB-PET showed that in both visual and quantitative analysis, PIB-PET had greater accuracy in differentiating AD patients from controls (Ng et al. 2007). However, in another study using automated ROI analyses, there was no difference in diagnostic accuracy between the two methods when analyzing the most representative areas of AD (hippocampus in FDG-PET, and middle frontal gyrus in PIB-PET) (Li et al. 2008). Devenand et al. (2010) found PIB-PET to be non-significantly more accurate in separating AD patients from healthy controls than FDG-PET, but the combination of both tracers conferred better accuracy, suggesting that the two techniques provide complementary information. A recent study compared the diagnostic performance of FDG-PET and PIB-PET in discriminating between AD and FTLD in patients around 65 years old. The sensitivity of PIB-PET was higher, but in the quantitative analyses FDG-PET exhibited superior specificity. (Rabinovici et al. 2011)

2.5 Environmental risk factors for Alzheimer's disease and dementia

2.5.1 General aspects of assessing risk factors for dementia

AD and VaD become increasingly prevalent with advancing age, and especially AD seems to be more common in women. (Lobo et al. 2000) An important potentially modifiable risk factor for dementia and especially for AD is a low educational level, and this association is not explained by different socioeconomic status, cardiovascular characteristics, or lifestyle factors between individuals with different educational backgrounds (McDowell et al. 2007; Ngandu et al. 2007).

As stated above, the pathological changes in AD are considered to precede clinical manifestation by 10-15 years (Jack et al. 2011; Perrin et al. 2009), and no treatment available today is able to terminate disease progression. In addition, it is possible that some risk or lifestyles factors could be modified by an early phase of cognitive decline, and therefore potential associations between these factors and dementia risk might be

obscured in studies without adequately long follow-up times. Hence, it would be very beneficial if the midlife risk factors for dementia could be identified. There has been increasing interest in the association between midlife cardiovascular risk factors and later dementia risk, and this review of the literature regarding these associations will be limited to long-term follow-up studies involving subjects who were middle aged at baseline. In addition, the review will be limited to the risk factors assessed in the current study.

Heavy alcohol drinking is known to cause multiple neurological disorders, including the cognitive disorders of alcohol related dementia (Oslin et al. 1998) and Korsakoff's syndrome. There has also been much interest in the effects of more moderate alcohol consumption on the risk of dementia and cognitive decline (for review, see Anstey et al. 2009; Peters et al. 2008). In contrast to cardiovascular factors, few studies have assessed the effects of midlife alcohol consumption on the dementia risk, and therefore studies on the relationship between drinking and dementia risk with follow-up times of over five years will be discussed.

2.5.2 Alcohol consumption

Yoshitake et al. (1995) followed 828 Japanese subjects older than 65 years and non-demented at baseline for seven years. The prevalence of dementia was examined with a two-step screening procedure at the end of the follow-up and using a daily monitoring system. Revised third edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-III-R), NINCDS-ADRDA and NINDS-AIREN criteria were used for dementia, AD and VaD, respectively. In the multivariate analysis, those subjects consuming alcohol at baseline were more likely than non-drinkers to develop VaD (OR 3.9) but not AD during follow-up. The Kungsholmen Project consists of at least 75 years old Swedish subjects free of dementia at baseline. A total of 402 individuals were followed for almost six years, and incident dementia and AD cases were detected during the whole follow-up using DSM-III-R and NINCDS-ADRDA criteria. Light to moderate drinkers (1-21 drinks per week for men, 1-14 drinks per week for women) had a decreased risk of dementia and AD when adjusting for age, sex, education, smoking, institutionalization, and baseline cognitive function (relative risk ratio [RR] 0.5 for both). Excessive drinkers were excluded from the analysis due to the limited number of subjects. (Huang et al. 2002)

Ruitenberg et al. (2002) studied 5 395 individuals of the Rotterdam Study aged on average 68 years at baseline with a mean follow-up time of six years. The dementia diagnosis was based on a three-step protocol including clinical examination, or on linkage to medical records. DSM-III-R, NINCDS-ADRDA and NINDS-AIREN criteria were used. The consumption of 1-3 drinks per day was associated with a decreased risk of dementia when compared to non-drinkers (hazard ratio [HR] 0.58, adjusted for multiple confounders), but no significant association was found for drinking up to one drink per day or at least four drinks per day. However, only five subjects who developed dementia reported drinking at least four drinks per day. The finding was similar for VaD, but for AD no significant HRs were found. There was no evidence that the risk reduction

depended on sex or the type of beverage. In ApoE ε4 carriers, drinking from one drink per week up to three drinks per day was associated with a reduced dementia risk as compared to non-drinkers, whereas no significant associations were found in non-carriers.

A nested case-control study with a six-year follow-up of the Cardiovascular Health Study examined 373 incident cases of dementia and an equal number of non-demented controls. AD and VaD were diagnosed by a consensus of neurologists and psychiatrists using NINCDS-ADRDA and State of California Alzheimer's Disease Diagnostic and Treatment Centers criteria, respectively. Baseline alcohol consumption of 1-6 drinks per week was found to be protective of dementia and AD when compared to non-drinkers (OR 0.46 and 0.43, respectively). Drinking at least 14 drinks per week was not associated with any statistically significant increase in dementia risk. When stratified by gender, the association between alcohol consumption and dementia was generally inverse in women and U-shaped in men. In analyses stratified according to ApoE status, significant associations were seen only in ApoE non-carriers. (Mukamal et al. 2003)

2 950 initially non-demented subjects aged at least 65 years from the Personnes Agées QUID (PAQUID) cohort were followed for eight years, and moderate drinkers were found to have a smaller risk of incident dementia as compared to non-drinkers (RR 0.56) (Larrieu et al. 2004). Similarly, 1 489 subjects of the Cognitive Function and Aging Study aged 65 years and above at baseline were followed for up to six years, and subsequently screened for dementia using Automated Geriatric Examination for Computer Assisted Taxonomy which detects dementia as diagnosed by DSM-III-R criteria. After adjusting for age, sex, social class and education, there was no difference in the dementia risk between ever and never drinkers. (Yip et al. 2006)

Two studies have followed elderly individuals for over ten years. A total of 2 805 at least 60-year-old participants of the Dubbo Study free of dementia at baseline were prospectively followed for up to 16 years. Dementia cases were detected during follow-up only through continuously monitoring hospitalization, nursing home admission and death records, which means that mild cases of dementia were probably missed. As compared to non-drinkers, those drinking 1-7 drinks per week at baseline had a decreased dementia risk (HR 0.70), as did individuals drinking 8-14 or 15-28 drinks per week (HR 0.65 and 0.40, respectively). (Simons et al. 2006) A total of 1 709 at least 65-year-old individuals in the Copenhagen City Heart study who had reported their alcohol consumption 15 years earlier were screened for cognitive impairment, and subjects with suspected dementia were examined more thoroughly. The diagnoses of dementia and VaD were based on DSM-III-R criteria and that of AD on NINCDS-ADRDA criteria. In a case-control analysis including 83 subjects with dementia and 1 626 controls, 15-21 drinks per week was associated with an increased dementia risk in the univariate analysis compared to 1-7 drinks per week (OR 2.26), but the association lost statistical significance in the multivariate analysis. Importantly, less than one drink per week or 8-14 drinks per week were not associated with any increased dementia risk. In additional multivariate analyses, monthly and weekly drinking of wine were associated with a

reduced dementia risk compared to never/hardly ever drinking (OR 0.43 and 0.33, respectively). In contrast, monthly beer consumption was associated with a significant increase in the dementia risk (OR 2.28). (Truelsen et al. 2002)

The studies described above have had follow-up times ranging from six to 16 years, but the subjects were elderly already at baseline. Hence, especially in the shortest studies, a proportion of subjects developing dementia during follow-up were likely to have exhibited cognitive deficits at baseline and this could well have affected their alcohol consumption, and thus obscure the association between dementia and drinking. To assess risk factors prior to the onset of pathological processes and the appearance of even slight cognitive problems, midlife alcohol consumption needs to be assessed. However, as of today, very few studies have examined the association between midlife drinking and risk of dementia.

Järvenpää et al. (2005) studied 554 MZ twins of the Finnish Twin Cohort that are also part of the cohort of studies **III** and **IV** presented here. Drinking was assessed using a self-report questionnaire and cognitive function was evaluated on average 25 years later using a validated telephone interview. Light ($\leq$ 3 drinks per week) or moderate (4-7 drinks per week for women and 4-14 drinks per week for men) drinkers did not differ from non-drinkers in their dementia risk when controlling for age, sex and education. Heavy drinkers had a point estimate indicating an increased dementia risk (non-significant OR 2.4), but statistical significance was not achieved, probably because only six demented subjects reported heavy drinking in midlife. It is notable that when controlling for total consumption of alcohol, at least monthly binge drinking was associated with an increased dementia risk (OR 5.6).

A total of 1 018 middle aged subjects at baseline (mean age 48 years) residing in two towns in Eastern Finland were followed for 23 years in the Cardiovascular Risk Factors, Aging and Dementia (CAIDE) study. The dementia diagnosis was based on DSM-IV criteria and AD diagnosis on NINCDS-ADRDA criteria. Neither non-drinkers nor frequent drinkers (defined as drinking several times a month) differed from infrequent drinkers (drinking less than once a month) in the dementia risk when controlling for age, sex and education. The interaction between drinking and ApoE genotype was significant, and in stratified analyses the risk of dementia increased with increasing alcohol consumption in ε4 carriers (OR 7.07 in frequent drinkers compared to non-drinkers). In non-carriers, the dementia risk did not differ between the various drinking categories (non-significant OR 0.45 for infrequent drinking and 0.75 for frequent drinking). (Anttila et al. 2004)

Finally, a group of 1 462 38-60 year-old women of the Prospective Population Study of Women were followed for up to 34 years for incident dementia. The dementia assessment was based on neuropsychiatric examinations and medical records, and DSM-III-R criteria and NINCDS-ADRDA criteria were applied for dementia and AD, respectively. Baseline consumption of either wine or beer was not associated with any significantly different HR compared to non-drinkers in a model adjusting for multiple confounding factors, but drinking spirits was a borderline significant risk factor for

dementia. In contrast, when including alcohol consumption data throughout the follow-up period, wine consumption was found to be protective of dementia (HR 0.56). (Mehlig et al. 2008)

To conclude, light to moderate drinking seems to be protective of dementia as compared to abstinence in studies with follow-up times up to 16 years, whereas findings for AD are somewhat inconsistent. In contrast, total alcohol consumption in midlife has not been associated with later dementia or AD risk, but two out of three studies assessing this association have utilized only binomial (Mehlig et al. 2008) or frequency assessment of drinking (Anttila et al. 2004).

The reduced dementia risk associated with drinking might not be directly attributable to any protective effect of alcohol. Non-drinkers differ from current drinkers in numerous lifestyle and demographic characteristics, including differences in dietary habits, body mass index (BMI) and physical activity (Barefoot et al. 2002; Fillmore et al. 1998), so the risk reduction associated with drinking may be linked to other factors influencing health. Some studies have hinted that wine may be especially protective (Mehlig et al. 2008; Truelsen et al. 2002). This could be caused by specific components in wine, but it is also possible that wine consumption reflects other factors that are associated with good health (Barefoot et al. 2002; Tjonneland et al. 1999), and drinking patterns seem to differ with beverage preferences (Gronbaek et al. 2000). Both of these phenomena potentially confound the results.

It is notable that none of the studies described above have reported any increased dementia risk associated with heavy drinking. This could be attributed to a number of reasons. Most studies have used non-drinkers as the reference group, and therefore might have missed the harmful effect of heavy drinking as compared to more moderate drinking. Additionally, in some studies the number of heavy drinkers has been low (Järvenpää et al. 2005; Ruitenberg et al. 2002), heavy drinkers have been excluded from analyses (Huang et al. 2002), or their results have not been reported (Simons et al. 2006). Furthermore, none of the studies examining the association between alcohol consumption in older age and dementia risk have assessed the effects of different drinking patterns.

2.5.3 Midlife cardiovascular risk factors

2.5.3.1 Hypertension

Multiple large cohort studies have assessed the association between midlife hypertension and dementia risk. The Honolulu-Asia Aging Study (HAAS) followed 3 555 men living on Hawaii in 1965. Cardiovascular risk factors were assessed when the subjects were 45-68 years old, and screening for dementia took place on average 25 years later. After screening, the final dementia, AD and VaD diagnoses were based on DSM-III-R, NINCDS-ADRDA and criteria proposed by the California Alzheimer's Disease and Treatment Centers, respectively. After adjusting for age and education, an increase of one standard deviation (SD) in diastolic (11.0 mmHg) or systolic blood pressure (18.5

mmHg) was not associated with any significant increase in the dementia risk. (Kalmijn et al. 2000)

Additional analysis of the HAAS cohort using categorical blood pressure as a variable did detect a significant association between hypertension and dementia risk. When controlling for age, education, ApoE genotype, smoking and alcohol consumption, it was found that high untreated diastolic blood pressure ($\geq$ 95 mmHg) in midlife was associated with a RR of 4.32 for dementia and 4.61 for AD as compared to normal diastolic blood pressure (80–89 mmHg). Similarly, subjects with high untreated systolic blood pressure ($\geq$ 160 mmHg) had a RR of 4.85 for dementia and 10.7 for VaD when compared to subjects with a normal level (110–139 mmHg), but the risk increase for AD was non-significant. In subjects treated with antihypertensives, only the risk for VaD associated with high systolic blood pressure was statistically significant. (Launer et al. 2000) In further analyses, those subjects with high systolic blood pressure displayed an increased risk of poor cognitive function regardless of ApoE genotype. However, the combined effect of ε4 allele and high systolic blood pressured was estimated to be greater than the sum of their individual effects, even though the interaction between ApoE genotype and blood pressure was statistically non-significant. (Peila et al. 2001)

In the CAIDE study, elevated midlife systolic blood pressure ($\geq$ 160 mmHg) was found to be a significant risk factor for AD with an OR of 2.8, whereas high diastolic blood pressure ($\geq$ 95 mmHg) had a non-significant OR when controlling for multiple confounders. In addition, subjects who developed AD were more likely to have used antihypertensive medication in midlife. (Kivipelto et al. 2001) In subsequent analyses, the risk associated with high systolic blood pressure was unaffected by controlling also for ApoE status, and no significant interactions were found between ApoE status and systolic blood pressure in the risk for AD (Kivipelto et al. 2002).

A prospective study examining 1 774 Japanese subjects from the Adult Health Study of the Radiation Effects Research Foundation estimated midlife blood pressure in 1965-1968. Cognitive function was subsequently assessed in 1992-1997 using a two-step screening procedure. Dementia and its subtypes were diagnosed according to DSM-IV criteria. After adjustment for age, sex and education, it was found that 10 mmHg increments in systolic blood pressure were associated with significant ORs of 1.04 and 1.03 for VaD and AD, respectively, but in the multivariate analysis, the risk increase for AD became non-significant (Yamada et al. 2003)

Whitmer et al. (2005) studied a multiethnic cohort of 8 845 individuals from the Kaiser Permanente Medical Care Program of Northern California. The subjects had participated in voluntary multiphasic health checkups when they were on average 42 years old. Mean follow-up time from baseline to dementia diagnosis, death or end of follow-up was 26 years. Dementia was ascertained through medical records and included diagnoses of dementia, memory impairment, AD, VaD and non-specified dementia. Midlife hypertension (blood pressure $\geq$ 140/90 mmHg, physician-diagnosed hypertension or reported use of antihypertensive medication) was associated with a significant HR of 1.24 when controlling for age, sex, race and education.

There is rather robust evidence indicating that midlife hypertension increases dementia risk when compared to normotensive values, and in two populations this association has been shown to be independent of ApoE genotype (Kivipelto et al. 2002; Peila et al. 2001). There is limited evidence that the risk increase is confined to subjects with untreated hypertension (Launer et al. 2000). The cut-off points for hypertension has been 90-95 mmHg for diastolic and 140-160 mmHg for systolic blood pressure. Studies analyzing blood pressure as a continuous variable have reported inconsistent results (Kalmijn et al. 2000; Yamada et al. 2003).

2.5.3.2 Hypercholesterolemia and dyslipidemia

Prospective cohort studies have also addressed the association between midlife serum cholesterol level and later dementia or AD risk. A study of 444 men aged 40-59 years at baseline from the Finnish cohorts of the Seven Countries Study assessed participants' cognitive function 30 years later. A two-stage screening procedure to detect dementia cases was used, and the final dementia diagnosis followed DSM-III-R criteria. After controlling for age and ApoE genotype, an OR of 3.1 was found for AD in those subjects with serum cholesterol concentration $\geq$ 6.5 mmol/l when compared to those with concentration < 6.5 mmol/l. (Notkola et al. 1998)

The association between blood lipids and dementia has also been studied in the same cohorts as the association between hypertension and dementia. Findings from the HAAS showed that an increase of one standard deviation in serum triglycerides (2.05 mmol/l) was associated with a significantly increased risk of dementia (RR 1.26), whereas a similar increase in serum total cholesterol (0.92 mmol/l) was not associated with any significantly increased risk (non-significant RR 1.10). (Kalmijn et al. 2000) In contrast, a high serum total cholesterol level ($\geq$ 6.5 mmol/l) was a significant risk factor for AD in the CAIDE study (OR 2.2) (Kivipelto et al. 2001). The finding was unaffected by controlling for ApoE genotype (Kivipelto et al. 2002). In addition, a retrospective analysis of the Kaiser Permanente Medical Care Program with 9 844 participants reported that a high total cholesterol level ($\geq$ 6.2 mmol/l) was associated with an increased AD risk three decades later (HR 1.57) as compared to a desirable cholesterol level (<5.2 mmol/l) when controlling for multiple confounding factors. Belonging to the two highest cholesterol level quartiles was also a significant risk factor for AD (HR 1.31-1.58). (Solomon et al. 2009)

A more recent study examined 1 462 participants of the Prospective Population Study of Women in Sweden. The first assessment of risk factors was done between ages 38 and 60, and the cohort was followed for up to 32 years. No significant association was found between a high baseline total cholesterol level (>6.5 mmol/l) and dementia or AD risk among subjects alive in 2001 when adjusting for confounders. In contrast, a declining total cholesterol level from baseline increased the risk of dementia. (Mielke et al. 2010) Furthermore, in the Finnish cohorts of the Seven Countries Study, as well as in the CAIDE study and HAAS it was noted that a declining cholesterol level from midlife was associated with an increased risk of AD, VaD or dementia (Notkola et al. 1998; Solomon

et al. 2007; Stewart et al. 2007). Similar findings have also been reported for BMI (Gustafson et al. 2009).

To summarize, most studies have shown an increased AD or dementia risk to be associated with serum total cholesterol levels above 6.2-6.5 mmol/l, even though this association was not seen in Swedish women or in the HAAS. In the CAIDE study, the risk increase was shown to be unaffected by adjusting for ApoE status. However, none of the studies described above have assessed the contribution of either low or high-density lipoproteins to the dementia risk, and only in HAAS has the effect of triglyceride levels been evaluated.

2.5.3.3 Overweight and obesity

The earliest findings linking midlife obesity to dementia risk emerged from HAAS, which showed that every single SD increase (2.9 kg/m^2) in BMI (calculated as weight in kilograms divided by the square of height in meters) was associated with an elevated risk of dementia, as was a corresponding increase in subscapular skinfold thickness (RR 1.21 for both) (Kalmijn et al. 2000). More recent studies have assessed this association in more detail. Rosengren et al. (2005) followed 7 402 Swedish men for 25-28 years. The subjects were on average 51 years old at baseline. Dementia cases were detected through linkage to the Swedish national register which provided information on cause of death, and to the Swedish Hospital Discharge Register, meaning that only moderate to severe dementia cases were probably identified. After adjusting for confounders, midlife BMIs of 27.50-29.99 kg/m^2 and ≥30 kg/m^2 were associated with increased risks of dementia when compared to a BMI of 20-22.49 kg/m^2 (HR 1.69 and 1.84, respectively).

The association between dementia and BMI was assessed in 10 136 subjects from the Kaiser Permanente of Northern California. The subjects were on average 42 years old at baseline and were followed for a mean of 36 years. Being overweight (BMI 25-29 kg/m^2) or obese (BMI ≥ 30 kg/m^2) was associated with an increased risk of AD when adjusting for multiple confounders (HR 2.09 and 3.10, respectively). Both characteristics were also significant risk factors for VaD (HR of 1.95 and 5.01, respectively). Additionally, there was a linear correlation between midlife BMI and later AD and VaD risk, so that even BMI 22.5-25 kg/m^2 increased AD risk compared to BMI < 22.5 kg/m^2. (Whitmer et al. 2007) In the CAIDE study, midlife obesity (BMI > 30 kg/m^2) was associated with a significantly increased risk of dementia after adjusting for age, sex and education (OR 2.44). However, the statistical significance was lost when also controlling for multiple cardiovascular risk factors and ApoE status. Findings for AD were statistically non-significant. (Kivipelto et al. 2005)

In the Prospective Population Study of Women in Sweden described above, a waist-to-hip ratio greater than 0.80 in midlife was a risk factor for dementia when controlling for age, sex, systolic blood pressure, cholesterol and triglycerides (OR 2.22). In contrast, no significant risk increase was associated with BMI ≥ 25 kg/m^2 at baseline. However, the study included only women, and the authors noted that the study population had relatively low average BMI of 24.1 kg/m^2 at baseline, which could explain the negative

findings. (Gustafson et al. 2009) A recent study of 8 534 subjects of the Swedish Twin Registry assessed the association between self-reported BMI at midlife (mean age 43 years) and dementia risk on average 30 years later. Cognitive impairment was first screened for by a telephone interview, and dementia, AD and VaD diagnoses were confirmed through a clinical work-up using DSM-IV, NINCDS-ADRDA and NINDS-AIREN criteria, respectively. Midlife overweight (BMI 25-30 kg/m^2) was associated with an increased risk of dementia and AD when controlling for age, sex and education (OR 1.37 and 1.41, respectively). In addition, obesity (BMI > 30 kg/m^2) increased the risk of dementia, AD and VaD (OR 3.01, 2.87 and 4.38, respectively). The results were unaffected by controlling for cardiovascular disorders. A matched case-control analysis was conducted for twin pairs discordant for dementia, and neither being overweight nor obese was a significant risk factor for dementia in these analyses. (Xu et al. 2011)

2.5.3.4 Physical inactivity

In the Adult Health Study of the Radiation Effects Research Foundation described above no significant association was found between dementia risk and a physical activity index which took into account both occupational and leisure activities. (Yamada et al. 2003) In contrast, in the CAIDE study, participating in leisure-time activity at least twice a week decreased the dementia and AD risk compared to those participating less than once a week (OR 0.45 and 0.34, respectively). Additional adjustments for ApoE genotype, midlife body-mass index and cardiovascular disorders did not affect the results, but in subgroup analyses, the association seemed more significant in ε4 carriers. (Rovio et al. 2005). Interestingly, a similar analysis of the CAIDE cohort found no association between midlife occupational or commuting physical activity and later dementia or AD risk (Rovio et al. 2007).

In an unmatched case-control analysis of 3 134 Swedish twins, both light and regular exercise in midlife were associated with a reduced risk of dementia compared to hardly any exercise when controlling for age, sex and education (OR 0.61 and 0.32, respectively). Similar findings were found for AD, and the results remained similar even when adjusting for multiple cardiovascular factors. Assessment of dementia was similar to the study on BMI described above. In a co-twin control analysis including twin pairs discordant for dementia those co-twins exercising less seemed to be more likely to develop dementia or AD, although the association did not reach statistical significance. (Andel et al. 2008) In a similar co-twin control analysis of American twins in the Duke Twins Study of Memory in Aging, 147 male twin-pairs were studied. Midlife physical activity was assessed with a self-report questionnaire at baseline. Dementia was ascertained 23-38 years later through a three-stage screening protocol, and the final dementia diagnosis was based on clinical examination using DSM-III-R criteria. No association was found between physical activity in midlife and later dementia risk. (Carlson et al. 2008)

A total of 4 942 subjects in the Age Gene/Environment Susceptibility - Reykjavik Study were followed for an average of 26 years. All participants were administered a battery of cognitive tests and a three-step procedure was used to ascertain dementia according to

DSM-IV criteria. Subjects who reported exercising for up to five hours per week were less likely to develop dementia than subjects reporting no weekly exercise (OR 0.59 when adjusting for age, sex and education). The OR associated with more than five hours of weekly exercise was similar but statistically non-significant. The findings were unaltered by additional adjustment for BMI, blood pressure, smoking and cholesterol level. (Chang et al. 2010)

The possible protective effect of physical activity seems to be limited to leisure-time exercise, and in the CAIDE study this effect was shown to be unaffected by controlling for ApoE genotype (Rovio et al. 2005). However, the protective level of physical activity has been described as being attributable to light and regular exercise (Andel et al. 2008), exercising at least twice a week (Rovio et al. 2005), and up to five hours of weekly exercise (Chang et al. 2010).

2.5.3.5 Clustering of cardiovascular risk factors

Even though multiple studies have assessed the effects of individual midlife cardiovascular risk factors on the risk of dementia, far fewer studies have assessed the additive effects of multiple such risk factors. In the HAAS cohort, a statistical z score including random postload glucose level, systolic and diastolic blood pressures, BMI, subscapular skinfold thickness, total cholesterol, and triglycerides was constructed. The score ranged from approximately -13 to 13, and every one-unit increase was associated with an increased dementia risk when adjusting for age and education (RR 1.06), but the association with AD was statistically non-significant. When subjects were divided into quartiles based on the score, there was a gradual and significant increase in the dementia risk with increasing scores. (Kalmijn et al. 2000) Whitmer et al. (2005) calculated a composite cardiovascular disease risk score based on a modified version of the Framingham Cardiovascular Risk Score including hypertension, diabetes, hypercholesterolemia and smoking in midlife for the subjects in the Kaiser Permanente Medical Care Program of Northern California. As compared to subjects with none of the risk factors, the HR for dementia ranged from 1.27 in subjects with one risk factor to 2.37 in subjects with all four risk factors after adjusting for age, race, education and sex.

The CAIDE study demonstrated that subjects with both hypertension and hypercholesterolemia at midlife are at a higher risk of developing AD than subjects with only one of the two risk factors (Kivipelto et al. 2001). Subsequent analysis detected no significant interactions between ApoE genotype, total cholesterol and systolic blood pressure in the risk for AD and VaD, but the combinations of these risk factors did increase the risk in an additive manner (Kivipelto et al. 2002). These findings led to the development of a risk score for predicting dementia risk 20 years in the future (Kivipelto et al. 2006). The included risk factors were age, education, sex, systolic blood pressure, BMI, total cholesterol and leisure time physical activity. In an alternative score ApoE genotype was also included. Scores for individual risk factors were estimated using a logistic regression model. Subsequent testing of the risk scores revealed areas under curve (AUC) of 0.769 and 0.776 in receiver-operating characteristic (ROC) analyses for the score with and without ApoE genotype, respectively. However, the score was tested

on the same population in which it was developed, possibly resulting in a form of circular evidence.

3. AIMS OF THE STUDY

The aims of this study were twofold. Firstly, the contribution of genetic factors to the changes in cerebral glucose metabolic rate associated with AD was studied in twin pairs discordant for AD. Secondly, possibly modifiable midlife risk factors for later cognitive impairment and their interactions with genetic factors were evaluated. More specifically, the practical objectives were as follows:

I   To assess regional brain metabolism in non-demented subjects who have a dizygotic co-twin suffering from AD applying similar methods to those used in a previous study on discordant monozygotic twin pairs (Järvenpää et al. 2003).

II   To assess brain metabolism in non-demented subjects who have a mono- or dizygotic co-twin suffering from AD using advanced automated voxel-level methods without any *a priori* assumptions about the topography of possible changes.

III   To analyze whether midlife alcohol consumption and drinking pattern are predictors of cognitive impairment in later life in Finnish twins.

IV   To analyze the effect of multiple individual midlife cardiovascular risk factors and their additive effects on later risk of cognitive impairment, and to evaluate a previously suggested risk score said to predict the risk of dementia 20 years later (Kivipelto et al. 2006).

V   To assess whether the possible associations between midlife drinking or cardiovascular risk factors and cognitive impairment risk depend on genetic or familial factors.

4. SUBJECTS AND METHODS

4.1 Subjects

The Older Finnish Twin Cohort was established in 1974. Finnish twin pairs were identified from the Central Population Registry of Finland, and same-sex twin pairs born prior to 1958 with both co-twins alive in 1967 were included in the cohort. As no selection criteria except age were initially used, the cohort is in effect a population sample. The cohort was set up with the permission of the Ministry of Social Affairs and Health and has been approved by the data protection ombudsman. Data on the twins are collected for research purposes only, and all questionnaire and registry data are obtained in coded format based on individual twin and family research codes. (Kaprio et al. 1978) The cohort included a total of 13 888 twin pairs of known zygosity at the beginning of prospective follow-up in August 1975. Twin zygosity was determined with a validated questionnaire (Sarna et al. 1978), and in a subsample, genetic markers have been used to ascertain zygosity. Three surveys of the whole cohort have been carried out. The first mailed questionnaire was carried out in 1975 and follow-up questionnaires in 1981 and 1990. The response rates to the questionnaires have been high (89 and 84 % for the first two questionnaires, respectively). The questionnaires assessed the twins' demographic, social, environmental, medical and lifestyle characteristics extensively. (Kaprio and Koskenvuo 2002)

All twins aged 65 or older were asked to participate in TELE, a telephone interview evaluating cognitive performance described in detail below. MZ twin pairs with both co-twins alive were interviewed between 1999 and 2001, and DZ twin pairs between 2003 and 2007, irrespective of co-twin status. Twins with questionnaire data from 1981 participating in the TELE interview formed the study population of studies **III** and **IV** evaluating the associations between midlife alcohol consumption or cardiovascular risk factors and cognitive impairment risk in later life. This study population includes MZ twins from a previous study on the association between midlife alcohol consumption and later dementia risk (Järvenpää et al. 2005).

Twin pairs appearing to be discordant for dementia according to TELE were asked to participate in a neuropsychological examination, MRI imaging and FDG-PET studies in Turku PET Centre. Seven such MZ and nine DZ twin pairs together with 13 unrelated healthy controls matched to the twins at group level for age and sex formed the study groups in studies **I** and **II**. The zygosities of the MZ twins included in study **II** were confirmed using ten highly polymorphic genetic markers. The MZ twins in study **II** have previously been included in a FDG-PET study by Järvenpää et al. (2003).

All study protocols were approved by the Joint Ethical Committee of University of Turku and Turku University Hospital. Informed consent was obtained from all participants before the telephone interview and PET imaging.

4.2 Assessment of demographic and risk factors (studies III and IV)

Subjects' educational level was assessed in the 1981 questionnaire using nine response categories. For the purpose of studies **III** and **IV,** these data were converted into years of formal schooling and classified into four educational levels: up to six years of formal schooling, 7-12 years of formal schooling, at least 13 years of formal schooling and other education.

The quantity of total alcohol consumption was assessed with questions on the consumption of specific beverages during an average week (beer and wines) or month (spirits). A seven-point scale was used for each beverage ranging from no use to over 48 bottles of beer, over 10 bottles of wine and over 20 bottles of spirits. The reported measures were then converted to estimates of alcohol grams by summing up all beverage types, and subsequently converted to drinks per week assuming that one drink contained 12 grams of pure ethanol. The subjects were then classified into non-drinkers, light drinkers (alcohol intake > 0 drinks/week and ≤ 3 drinks/week), moderate drinkers (alcohol intake > 3 and ≤ 7 drinks/week for women, > 3 and ≤ 14 drinks/week for men) and heavy drinkers (> 7 drinks/per week for women, > 14 drinks/week for men) based on the 1981 questionnaire. The upper limit of the moderate drinker category is in line with the limit of low-risk consumption suggested by the National Institute of Alcohol Abuse and Alcoholism (National Institute of Alcohol Abuse and Alcoholism 2010). Additionally, in the 1975 questionnaire subjects were asked to report whether they had drunk more at some previous point in their lives.

Drinking pattern was assessed with questions on the consumption of large quantities of alcohol at the one and same occasion. The number of passing outs (pass-outs), or loss of consciousness after and because of heavy alcohol consumption during the prior year was assessed in the 1981 questionnaire. Subjects were classified into those reporting no pass-outs, 1-2 pass-outs and at least three pass-outs. Binge drinking was assessed both in 1975 and 1981, and was defined as drinking more than five bottles of beer, one bottle of wine, half a bottle of spirits, or an equivalent amount of other beverages on the one and same occasion on at least a monthly basis. Subjects were classified into those reporting binge drinking in neither 1975 nor 1981, only in 1975 or 1981, and in both 1975 and 1981.

The assessment of midlife cardiovascular risk factors was based on the 1981 questionnaire. Because study **IV** aimed to evaluate a previously developed dementia risk score (Kivipelto et al. 2006), only the factors included in the score were examined and, thus, e.g. the role of diabetes was not assessed. BMI was calculated using self-reported weight and height, and subjects were classified according to a modified World Health Organisation proposal (World Health Organization 2011): lean or normal weight (BMI < 25 kg/m^2), overweight (BMI ≥ 25 kg/m^2 and < 30 kg/m^2) and obese (BMI ≥ 30 kg/m^2). Self-reported and measured BMI have been shown to correlate well (Korkeila et al. 1998).

The presences of hypertension and hypercholesterolemia were assessed with the question "Has a nurse or a doctor measured your blood pressure/Has your cholesterol level been measured during the last five years?" including the following answers: has not been measured, subject does not recall if has been measured, found normal, and found elevated. Blood pressure was considered high even if it had been found to be only slightly elevated. In addition, subjects who reported physician diagnosed hypertension or use of anti-hypertensive pharmaceuticals were included in the hypertensive group. Self-reported history of hypertension and use of anti-hypertensive pharmaceuticals have been shown to be reliable (Haapanen et al. 1997; Hernelahti et al. 1998).

Leisure time physical activity was assessed with a question on monthly frequency, mean duration and mean intensity of physical activity sessions. Subjects reporting exercise at least six times per month with a mean duration of at least 30 minutes and intensity corresponding at least vigorous walking were classified as conditioners, whereas subjects reporting no participation in leisure time physical activity were classified as sedentary. Other subjects were classified as occasional exercisers. To further assess the volume of physical activity, an activity metabolic equivalent (MET) index was calculated. This was achieved by assigning a multiple of resting metabolic rate to each assessed activity and then calculating the product of intensity × duration × frequency of activity. The MET index was determined as the sum of leisure MET hours per day. (Kujala et al. 1998) Subjects were then classified into MET index quartiles. In the case of missing physical activity information in 1981 questionnaire, data from the 1975 questionnaire was used.

To assess ApoE genotype, venous blood samples were collected at local health centres using a kit mailed to the subjects. Samples and consent forms were returned to the National Public Health Institute, Helsinki, Finland. ApoE was genotyped using an analysis of two single-nucleotide polymorphisms, rs429358 and rs7412 (SNPedia 2011) with success rates of 98.54 % and 98.28 %, respectively. If genotype was available for only one co-twin in a MZ twin pair, the same genotype was assumed for the other co-twin with the missing genotype data; in some pairs, both MZ twins were genotyped and confirmed to have the same genotype in each case. ApoE genotype was available for 1 611 (74 %) interviewed subjects with questionnaire data, and these subjects were classified into those without an ε4 allele and those with at least one ε4 allele.

4.3 Dementia risk score (study IV)

A score predicting an individual's dementia risk 20 years in the future has been developed based on the CAIDE study (Kivipelto et al. 2006). The score includes age, educational level, sex, systolic blood pressure, BMI, serum total cholesterol and physical activity. An alternative score also includes ApoE genotype. In order to validate the postulated risk score in the current study population, questionnaire data of study **IV** were re-categorized to match the suggested score as precisely as possible. Exactly the same categorizations could be utilized for age, sex, education and BMI. For hypertension and hypercholesterolemia the categorization described above were used, and for physical activity exercising at least six times per month with a mean duration of at least 30

minutes and intensity corresponding to at least vigorous walking was used as the cut-off point. Details of the originally suggested and modified risk score in study **IV** are shown in **Table 1**.

Table 1. Details of the original, previously suggested dementia risk score (Kivipelto et al. 2006) and the risk score modified for study **IV**. Score 1 includes age, educational level, sex, blood pressure, BMI and cholesterol level. Score 2 also includes the number of ApoE ε4 alleles. The table has been published in Original Publication IV.

Original risk score	Modified risk score	Score 1	Score 2
Age at baseline			
< 47 years	< 47 years	0	0
47-53 years	47-53 years	3	3
> 53 years	> 53 years	4	5
Education			
≥ 10 years	≥ 10 years	0	0
7-9 years	7-9 years	2	3
0-6 years	0-6 years	3	4
Sex			
Women	Women	0	0
Men	Men	1	1
Midlife blood pressure			
Systolic blood pressure ≤140 mmHg	Blood pressure found normal	0	0
Systolic blood pressure >140 mmHg	Blood pressure found high	2	2
Midlife body-mass index			
≤ 30 kg/m2	≤ 30 kg/m2	0	0
> 30 kg/m2	> 30 kg/m2	2	2
Midlife serum cholesterol level			
Total cholesterol ≤ 6.5 mmol/l	Cholesterol found normal	0	0
Total cholesterol > 6.5mmol/l	Cholesterol found high	2	1
Midlife physical activity			
Active*	Conditioner[O]	0	0
Inactive	Sedentary / Occasional exerciser	1	1
ApoE ε4 genotype			
No ε4 alleles	No ε4 alleles		0
1 or 2 ε4 alleles	1 or 2 ε4 alleles		2
Maximum score		15	18

* Active people reported leisure time physical activity at least twice a week
[O] Conditioners reported exercising at least six times per month with a minimun duration of 30 min and minimal intensity of vigorous walking

In order to achieve the subscores shown in **Table 1**, the CAIDE study first identified midlife vascular risk factors that were individually significant risk factors for dementia. Then these factors were simultaneously included in a multiple regression model, and the β coefficients of this final model were standardized so that the lowest subscore became equal to one. The individual subscores for the risk scores with and without the ApoE genotype were obtained from different regression analyses and, therefore, differ between the two scores. (Kivipelto et al. 2006) As the aim of study **IV** was to evaluate the suggested risk score, the originally suggested subscores were used. In order to calculate a dementia risk score for every subject, the subscores of individual risk factors were summed together. This yielded two different risk scores: the first does not include ApoE

genotype and has a maximum score of 15, and the second including ApoE genotype has a maximum score of 18.

4.4 Assessment of cognitive function

As stated above, at the end of follow-up, subjects were asked to participate in TELE, a self-report telephone interview assessing cognitive function (Gatz et al. 1995). Trained, experienced research nurses from the Department of Public Health in the University of Turku conducted the interviews. TELE has been shown to accurately distinguish patients with mild to moderate AD from healthy controls, and to correlate with dementia severity (Järvenpää et al. 2002). TELE has a maximum score of 20, and higher scores indicate better cognitive performance. For studies **III** and **IV,** a cut-off point of > 17.5 was used for intact cognitive function and < 16 for cognitive impairment. Subjects with a TELE score of 16-17.5 were classified as having mild impairment in their cognitive function (MICF). The sensitivity and specificity of the chosen cut-off points in separating AD patients from healthy controls are as follows: for the lower cut-off point 76.7 % and 100.0 %, and for the higher cut-off point 96.7 % and 69.2 % (Järvenpää et al. 2002). A diagnosis of dementia cannot be based solely on the TELE, and therefore the term cognitive impairment is used instead of dementia. TELE has not been validated for the assessment of mild cognitive deficits. Therefore, the MICF group was included mainly to increase the specificity in the cognitively intact and cognitively impaired groups, and the results of this group will not be discussed further here.

Twin pairs participating in the PET studies (**I** and **II**) were screened through the TELE interviews. Discordance of the participating twin pairs was then confirmed with an extensive neuropsychological test battery assessing episodic and semantic memory, attention, language and visuospatial abilities using the CERAD test (Morris et al. 1989), the revised Wechsler Memory Scale (Brinkman et al. 1983), parts of the revised Wechsler Adult Intelligence Scale (Wechsler 1981), a verbal fluency test, Trail Making Tests A and B (Reitan 1959), and the Stroop test (Houx et al. 1993). Two of the cognitively more impaired MZ co-twins fulfilled the older criteria for MCI (Petersen et al. 1999), whereas other cognitively more impaired co-twins were considered to have AD. Additionally, one of the cognitively less impaired MZ co-twins had MCI. For clarity, however, the cognitively more impaired co-twins are referred to as being demented and the less impaired as non-demented.

4.5 Acquisition and analysis of PET images (studies I and II)

4.5.1 PET and MRI imaging

All FDG-PET scans were performed with a GE Advance PET scanner (General Electric Medical Systems, Milwaukee,WI, USA) using the orbito-meatal line as the reference when positioning subjects. Thirty-five transaxial planes with a thickness of 4.35 mm were obtained, and transformed into a 128x128-pixel matrix using a planar Hann filter and an axial Ramp filter. Attenuation correction was done using two rotating rod

sources. The final resolution after reconstruction was 6-7 mm. Before imaging, cannulae were inserted for injection of the FDG bolus and for drawing arterialized venous blood samples. The scanning protocol consisted of an up to 55 minutes long dynamic PET scan with multiple blood samples being drawn for measurements of activity concentration in plasma. The mean injected activity was 257 ± 45.9 MBq. Furthermore, 1.5 tesla MRI scans were obtained for all subjects, and used to screen for possible brain lesions and as the anatomical reference in ROI defition.

4.5.2 Manual region of interest analysis (study I)

A manual ROI analysis was done for the DZ twin pairs and controls similarly to the previous study on the MZ twin pairs (Järvenpää et al. 2003). T1-weighted MRI scans were co-registered with summated PET scans using SPM2 (Wellcome Department of Imaging Neuroscience, University College London, London, UK). Bilateral ROIs were drawn individually for each subject to prefrontal cortex (Brodmann areas [BA] 9-10), medial frontal cortex (medial surface of BA 9), lateral temporal cortex, hippocampus, sensory motor cortex (BA 1–5), visual cortex (BA 17-18), caudate nucleus, putamen, thalamus and cerebellar cortex with Imadeus 1.20 (Forima Inc.,Turku, Finland) using the co-registered MRI scans as reference. rGMR was calculated for each ROI using Gjedde-Patlak plots with 0.52 as the lumped constant (Patlak and Blasberg 1985).

4.5.3 Statistical parametric mapping analysis (study II)

To further evaluate the extent of possible differences in cerebral GMR between the controls, non-demented MZ and DZ co-twins, and demented co-twins, a statistical parametric mapping (SPM) analysis was performed. Manual ROI based analysis of rGMR requires or at least implies a preconception about the topography of possible deficits or differences between groups. Additionally, an analysis of a large number of ROIs as independent measurements would require a strict statistical correction for multiple comparisons. SPM overcomes these issues by examining the whole brain space after spatial normalization to a common stereotactic space without any *a priori* hypothesis on the topography of differences, and by reporting corrected significance levels. The method has been clinically validated (Signorini et al. 1999).

In study **II**, parametric GMR images providing voxel-level data on glucose metabolism were calculated using Gjedde-Patlak plots as described above. The parametric images were spatially normalized using a ligand-specific template prepared with an MRI-aided procedure suggested before (Meyer et al. 1999). The FDG-PET template was generated from 14 FDG-PET scans of individuals not included in studies **I** or **II**. First, an individual's MRI scan was co-registered to his/her summated FDG-PET image, and then the co-registered MRI scan was normalized using a T1-weighted MRI template provided with SPM2. These normalization parameters were then applied to the summated FDG-PET image, and a FDG-PET template was calculated as a mean of these 14 summated, normalized images. This template was then averaged with its mirror copy and finally smoothed using an 8 mm Gaussian kernel.

The summated FDG-PET images of the subjects in study **II** were then co-registered to the FDG-PET template, followed by application of the same co-registration parameters to the subjects' parametric GMR images in order to finally obtain spatially normalized parametric GMR images. The normalized images were written using bilinear interpolation and smoothed using a 12 mm Gaussian kernel. The procedure resulted in PET images normalized to the Montreal Neurological Institute (MNI) space.

Using the individual normalized parametric GMR images two separate voxel-level one-way analyses of variance (ANOVA) were performed. The first included the controls, non-demented MZ co-twins and non-demented DZ co-twins to assess whether the non-demented MZ or DZ co-twins had reduced GMR compared to healthy, unrelated controls. In the second analysis, all the demented co-twins were compared to the controls, because it was assumed that the demented MZ and DZ co-twins manifest the same AD disease process, and because this pooling increased the statistical power. In the analysis including the non-demented co-twins $p < 0.05$ (corrected for multiple comparisons) and in the analysis including the demented co-twins $p < 0.01$ was considered statistically significant. In both analyses, clusters with volumes $\geq 1\,000$ voxels were considered significant.

4.5.4 Automated region of interest analysis (study II)

SPM analyses only provide the topography and statistical significances of possible differences between study groups, but no quantitative data on the differences. Therefore, automated ROI analyses were conducted as well using the normalized parametric GMR images described above. In contrast to a manual ROI analysis, operator induced error in defining ROIs separately for each subject is avoided as a common ROI set is used.

For the automated ROI analyses, a mean image of 11 spatially normalised T1-weighted MRIs was used as an anatomical reference, and ROIs were drawn bilaterally to prefrontal cortex (BA 10, 46), lateral temporal cortex (BA 20-22), parietal cortex (BA 19, 39), occipital cortex (BA 17-18), medial temporal lobe (including hippocampus and amygdala), caudate, putamen, thalamus and cerebellar cortex using Imadeus 1.20. rGMR was calculated similarly to the manual ROI analysis described above.

4.6 Statistical analyses

The manual and automated ROI analyses in studies **I** and **II** were done using SPSS 12.0 and 13.0, respectively (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant. In the manual ROI analyses of study **I,** asymmetry between hemispheres was tested using paired samples t-test, and since significant asymmetry was seen only in lateral temporal cortex, the mean rGMR values between the two hemispheres were used in the analyses. Comparison between groups was done with one-way ANOVA and Tamhane HSD post-hoc test as all rGMR values did not pass the homogeneity of variance test.

In the automated ROI analysis used in study **II**, the demented co-twins were pooled together, and the four remaining groups (controls, non-demented DZ co-twins, non-

demented MZ-cotwins and demented co-twins) were compared using repeated measures analysis with twin pair as a random variable to account for possible correlations between co-twins' rGMR values. rGMR values were analysed separately for both hemispheres in ROIs where asymmetry was significant in a paired samples t-test.

The statistical analyses of the studies on midlife risk factors in studies **III** and **IV** were done using Stata 11.1 (StataCorp, College Station, TX, USA), and $p < 0.05$ was considered significant. The analyses were done using robust estimators of variance and clustering to account for the twin structure of the study population (Williams 2000). RRs and 95 % CI were calculated for MICF and cognitive impairment in the analyses of individual risk factors using multinomial logistic regression. In addition to an unadjusted model, two multivariate models were tested. Model 1 controlled for age, sex and educational level. Model 2 also included ApoE status. All of the covariates are significant and established risk factors of AD and dementia (Farrer et al. 1997; Lobo et al. 2000; McDowell et al. 2007; Ngandu et al. 2007). The proportion of non-drinkers, heavy drinkers and binge drinkers differs in terms of age, sex and education (Helasoja et al. 2007; Jefferis et al. 2007). Smoking was not found to be an independent risk factor for cognitive impairment in the current study population, and therefore was not controlled for in the main analyses. In the analyses assessing the effects of binge drinking and pass-outs on cognitive impairment risk, total alcohol consumption was also controlled for as a categorical variable and non-drinkers were excluded.

In the alcohol analyses, light drinkers were used as the reference group. In the cardiovascular risk factor analyses, the reference groups were subjects with lean or normal BMI, normal blood pressure or normal cholesterol level, conditioners, and subjects in the highest MET index quartile. Possible interactions between sex, educational level or ApoE genotype and the assessed risk factors on cognitive impairment risk were tested in Model 1 using the Stata module xi3 with likelihood ratio tests (UCLA: Academic Technology Services, Statistical Consulting Group 2012).

In order to assess the suggested dementia risk scores, ROC analyses were conducted to obtain AUCs with 95 % CIs. The ORs of cognitive impairment were calculated for every risk score quartile using logistic regression. An additional logistic regression analysis was done using the scores as continuous variables in order to calculate predicted probabilities of cognitive impairment with 95 % CIs.

Co-twin control analyses allow for controlling of genetic and familial factors, and differences between co-twins can be attributed to different environmental factors. In studies **III** and **IV**, only twin pairs clearly discordant in cognitive function were included in these analyses excluding subjects with MICF. Assessed risk factors were turned into two- or three-level variables and ORs were calculated with conditional logistic regression without controlling for confounders. The web application Sampsize 0.6 was used to estimate the statistical power for these analyses (Glaziou 2005).

5. RESULTS

5.1 PET studies (studies I and II)

5.1.1 Characteristics of participating twins

The characteristics of the subjects in studies **I** and **II** are shown in **Table 2**. The groups did not differ in terms of age, and no difference was seen in Mini Mental State Examination (MMSE) scores between the non-demented MZ and DZ co-twins, or between the demented MZ and DZ co-twins.

Table 2. Characteristics of the subjects in the PET studies.

	Controls	Non-demented DZ co-twins	Non-demented MZ co-twins	Demented co-twins
Number of subjects	13	9	7	16
Number of women (%)	9 (69 %)	6 (67 %)	7 (100 %)	13 (81 %)
Mean age ± SD	73.3 ± 2.69	75.9 ± 5.42	75.1 ± 3.85	75.6 ± 4.66
Mean MMSE ± SD	28.8 ± 0.93	26.3 ± 1.12	26.0 ± 2.83	20.8 ± 4.39

5.1.2 Manual ROI analyses of discordant dizygotic twin pairs (study I)

Nine discordant DZ twin pairs and 13 controls were included in the manual ROI analyses. Compared to the controls, rGMR of the demented DZ co-twins was most severely reduced in caudate (23 % lower than in controls, p=0.03), hippocampus (20 %, p<0.01), putamen (17 %, p=0.01) and lateral temporal cortex (15 %, p<0.01). rGMR was also significantly reduced in all other ROIs except for sensory motor cortex, visual cortex, thalamus and cerebellar cortex. In contrast, there were no significant differences between the non-demented DZ co-twins and controls in any ROI. All absolute rGMR values are shown in **Table 3**.

In order to account for the possibly significant individual variation in absolute rGMR values between individuals, a comparison between the groups was conducted using rGMR values relative to sensory motor cortex. The sensory motor cortex was chosen because it is mainly spared by AD pathogenesis, and the rGMR reduction was smallest in the demented DZ co-twins in this ROI. The difference between the demented DZ co-twins and controls was greatest in caudate (19 % lower) and hippocampus (15 %). No differences were seen in medial frontal cortex, visual cortex, cerebellar cortex and thalamus. Overall, the differences between the demented co-twins and controls seemed smaller when comparing relative rather than absolute rGMR values. Again, the non-demented DZ co-twins did not differ from controls in relative rGMR in any ROI.

Table 3. Absolute rGMR values (mean ± SD) of the manual ROI analysis for controls, demented DZ co-twins and non-demented DZ co-twins. rGMR values are presented as µmol/ml/min and followed by relative values compared to controls in brackets.

	Controls (n = 13)	Demented co-twins (n = 9)	Non-demented co-twins (n = 9)
Prefrontal cortex	0.35 ± 0.03	0.29 ± 0.05 (84 %)*	0.36 ± 0.05 (104 %)
Medial frontal cortex	0.32 ± 0.03	0.29 ± 0.05 (89 %)	0.34 ± 0.05 (105 %)
Hippocampus	0.25 ± 0.02	0.20 ± 0.03 (80 %)*	0.25 ± 0.05 (102 %)
Lateral temporal cortex	0.32 ± 0.03	0.27 ± 0.03 (85 %)*	0.33 ± 0.04 (103 %)
Sensory motor cortex	0.32 ± 0.04	0.30 ± 0.04 (94 %)^{O}	0.34 ± 0.04 (106 %)^{O}
Visual cortex	0.35 ± 0.05	0.32 ± 0.06 (91 %)	0.39 ± 0.05 (113 %)
Cerebellar cortex	0.31 ± 0.04	0.27 ± 0.05 (88 %)	0.33 ± 0.04 (108 %)
Caudate	0.37 ± 0.04	0.29 ± 0.08 (77 %)*	0.38 ± 0.07 (101 %)
Putamen	0.39 ± 0.04	0.32 ± 0.04 (83 %)*	0.42 ± 0.09 (108 %)
Thalamus	0.36 ± 0.04	0.31 ± 0.07 (87 %)	0.38 ± 0.06 (105 %)

* Significant difference to controls ($p < 0.05$)
^{O} As the values of the controls did not pass the Shapiro-Wik test of normality, p values are calculated using the Mann-Whitney U test

5.1.3 SPM analyses of all discordant twin pairs (study II)

The first voxel-level ANOVA included the controls and non-demented MZ and DZ co-twins. In the contrast between the controls and non-demented MZ co-twins, the GMR values were lower bilaterally in inferior frontal, lateral temporal, parietal and medial temporal cortices in the non-demented MZ co-twins. In lateral temporal cortex, the difference seemed more significant in the left hemisphere. Additionally, GMR was lower in subcortical structures, including thalamus, putamen and right amygdala. According to the SPM analysis the significant voxels belonged to one highly significant cluster (corrected $p < 0.001$ at cluster-level). The cluster peak was located in the right cerebellum. In comparison, no significant clusters were identified in the contrast between the controls and non-demented DZ co-twins.

The second ANOVA included the controls and demented co-twins, and the demented co-twins had significantly lower GMR in frontal, parietal, lateral temporal and occipital cortices, medial temporal lobe as well as limbic structures and subcortical nuclei. In lateral temporal cortex, the reduction was more extensive in the left hemisphere. No differences were seen in primary sensory motor or visual cortices. SPM revealed a single highly significant cluster (corrected $p < 0.001$ at cluster-level) with its peak in the left hippocampus. The results of both SPM analyses are shown in **Figure 3** and **Table 4**.

In an additional SPM analysis comparing the non-demented MZ co-twins and the non-demented DZ co-twins, the non-demented MZ co-twins showed reduced GMR in a similar pattern to the comparison between the controls and non-demented MZ co-twins, but the differences were more extensive especially on the right hemisphere and posteroparietally when using similar thresholds (results not shown).
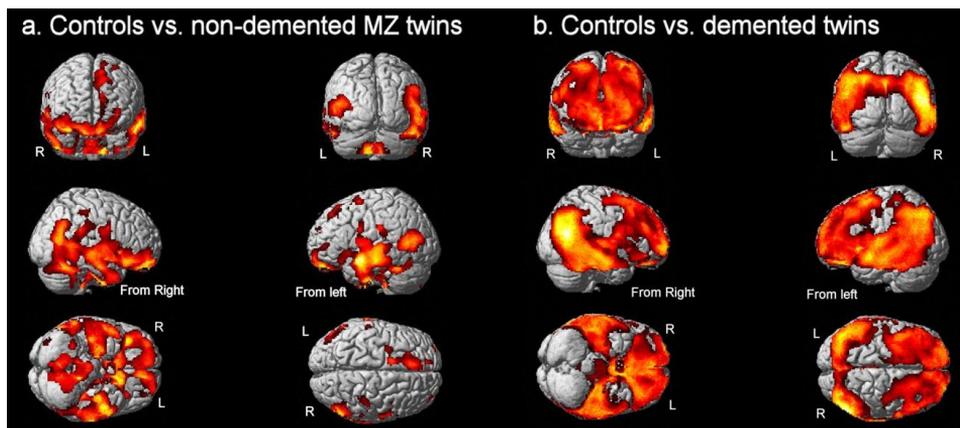
Figure 3. Cortical presentation of the voxel-level SPM analyses. Areas with significantly lower GMR values compared to controls are shown. The palette from red to yellow indicates increasing statistical significance. a) Analysis between the controls and non-demented MZ co-twins b) Analysis between the controls and demented co-twins. The figure has been published in Original Publication II. The comparison between the controls and non-demented DZ co-twins is not shown since no significant differences were found between the groups in any region.

Table 4. Cluster results of the SPM analyses. The upper part of each table shows the peak and subpeaks of the significant cluster with the relevant BA, T and Z statistics, and the corresponding MNI space coordinates. The second part shows the proportion (%) of selected subcortical structures inside the significant cluster. The table has been published in Original Publication II. The comparison between controls and the non-demented DZ co-twins is not shown since no significant differences were found between the groups in any region.

A. Controls vs. non-demented MZ co-twins

Cluster peak(cluster size 48179 voxels)	BA	T	Z	x, y, z (mm)
Right cerebellum	-	4.00	3.50	56, -64, -22
Cluster sub-peaks				
Left cerebellum	-	3.90	3.43	-4, -36, -28
Left middle temporal gyrus	21	3.81	3.37	-52, 2, -18
Righ temporal lobe, subgyral grey matter	21	3.64	3.24	44, -12, -12

Limbic and subcortical regions where difference was significant

	Pecentage of structure within the significant cluster	
Area	Left	Right
Amygdala	90.68	41.77
Hippocampus	20.14	45.08
Parahippocampal gyrus	42.56	36.79
Thalamus	51.03	55.26
Anterior Cingulate	15.40	3.88
Posterior Cingulate	27.91	13.33
Putamen	58.19	72.72
Caudatus	32.58	5.98

B. Controls vs. demented co-twins

Cluster peak(cluster size 99687 voxels)	BA	T	Z	x, y, z (mm)
Left hippocampus	20	6.86	5.18	-28, -14, -26
Cluster sub-peaks				
Left parahippocampal gyrus	36	6.85	5.17	-30, -18, -24
Left posterior cingulate	29	6.63	5.06	-12, -50, 10
Left parahippocampal gyrus	20	5.89	4.68	-22, -6, -28

Limbic and subcortical regions where difference was significant

	Percentage of structure within the significant cluster	
Area	Left	Right
Amygdala	94.41	94.94
Hippocampus	99.28	93.44
Parahippocampal gyrus	81.60	75.90
Thalamus	97.35	98.53
Anterior Cingulate	40.79	33.56
Posterior Cingulate	88.79	83.32
Putamen	99.17	89.21
Caudatus	97.58	83.84

5.1.4 Automated ROI analyses of all discordant twin pairs (study II)

Automated ROI analyses were performed to quantitatively assess the differences between the groups using a ROI set defined blind to the SPM analyses. Compared to the controls, the non-demented MZ co-twins had reduced rGMR values in right parietal cortex (15 % lower, p=0.02), left and right lateral temporal cortices (12 %, p<0.01 and

11 %, p=0.03, respectively), putamen (13 %, p=0.05), and thalamus (15 %, p=0.02). In contrast, no differences were found between the non-demented DZ co-twins and controls. The demented co-twins showed significantly reduced rGMR in every ROI except for the occipital and cerebellar cortices. All rGMR values are shown in **Table 5**. Two additional ROIs were defined guided by the SPM results between the non-demented MZ co-twins and controls. These included orbital frontal cortex and parieto-frontal cortex, and in right parieto-frontal cortex, the difference between the non-demented MZ co-twins and controls reached borderline significance (p=0.052). The results for these ROIs are shown at the end of **Table 5**.

Since in one MZ twin pair, the non-demented co-twin was suspected to have MCI, t-tests between the controls and non-demented MZ co-twins were conducted excluding this twin pair. Also in these analyses, the non-demented MZ co-twins had significantly lower rGMR values in right parietal cortex, left and right lateral temporal cortices, putamen and thalamus. As the MZ twins were all female, the analyses were repeated excluding men from all groups, leaving 9 controls, all 7 MZ twin pairs and 6 DZ twin pairs, and in these analyses, the non-demented MZ co-twins had significantly reduced rGMR in right parietal cortex, left and right temporal cortices, and thalamus.

Automated ROI analyses were also done using relative rGMR values with cerebellar vermis as the reference. In these analyses, neither the non-demented MZ nor DZ co-twins showed reduced relative rGMR in any ROI compared to controls, whereas relative rGMR was significantly reduced in the demented co-twins in frontal, lateral temporal, parietal, medial temporal and posterior cingulate cortex, as well as in caudate, putamen and thalamus.

Additional post-hoc analyses were done to compare the non-demented DZ co-twins with the non-demented MZ co-twins. In these analyses, the non-demented MZ co-twins had significantly lower rGMR values in the right parietal cortex, putamen and cerebellar cortex when adjusting for multiple comparisons. As the demented co-twins were pooled together in the main analyses, the demented MZ and DZ co-twins were not compared directly with SPM or in the main automated ROI analyses. However, the rGMR values were slightly higher in the demented MZ co-twins than in the demented DZ co-twin in each ROI, but this difference was significant only in the occipital cortex (independent samples t-test, two tailed p=0.02).

In an attempt to estimate the effect of genetic burden on cerebral glucose metabolism, the correlation was assessed between genetic load and rGMR in areas affected by AD. In accordance with an additive model, this genetic load was determined as 0 for controls, 0.5 for DZ non-demented co-twins and 1 for MZ non-demented co-twins. With respect to the areas typically affected by AD pathogenesis, only in lateral temporal cortex was a significant correlation seen (correlation coefficient -0.41, p=0.03).

Table 5. Absolute rGMR values (mean ± SD) of the automated ROI analyses for the controls, demented co-twins, non-demented MZ co-twins and non-demented DZ co-twins. rGMR values are presented as µmol/ml/min and followed by relative values compared to controls in brackets.

	Controls (n = 13)	Demented (n = 16)	Non-demented MZ (n = 7)	Non-demented DZ (n = 9)
Prefrontal cortex	0.33 ± 0.03	0.28 ± 0.06 (83 %)*	0.32 ± 0.05 (94 %)	0.34 ± 0.05 (101 %)
Parietal cortex	0.34 ± 0.02	0.27 ± 0.05 (81 %)*	0.29 ± 0.08 (86 %)†	0.35 ± 0.05 (104 %)
left	0.33 ± 0.02	0.27 ± 0.05 (82 %)*	0.29 ± 0.08 (86 %)	0.35 ± 0.05 (105 %)
right	0.34 ± 0.02	0.28 ± 0.04 (81 %)*	0.29 ± 0.07 (85 %)*†	0.35 ± 0.05 (103 %)
Lateral temporal cortex	0.31 ± 0.02	0.26 ± 0.03 (84 %)*	0.28 ± 0.03 (88 %)*	0.31 ± 0.04 (99 %)
left	0.31 ± 0.02	0.26 ± 0.04 (83 %)*	0.27 ± 0.04 (88 %)*	0.31 ± 0.04 (99 %)
right	0.31 ± 0.02	0.27 ± 0.04 (86 %)*	0.28 ± 0.03 (89 %)*	0.31 ± 0.05 (100 %)
Occipital cortex	0.32 ± 0.02	0.29 ± 0.05 (93 %)	0.30 ± 0.05 (93 %)	0.33 ± 0.04 (105 %)
left	0.32 ± 0.03	0.30 ± 0.05 (93 %)	0.30 ± 0.05 (94 %)	0.33 ± 0.04 (104 %)
right	0.32 ± 0.02	0.29 ± 0.05 (92 %)	0.29 ± 0.04 (92 %)	0.34 ± 0.04 (107 %)
Ant. cingulate	0.31 ± 0.03	0.26 ± 0.05 (86 %)*	0.28 ± 0.06 (91 %)	0.30 ± 0.05 (99 %)
Post. cingulate	0.38 ± 0.03	0.30 ± 0.05 (81 %)*	0.35 ± 0.09 (92 %)	0.39 ± 0.07 (104 %)
left	0.38 ± 0.04	0.30 ± 0.06 (81 %)*	0.34 ± 0.09 (91 %)	0.40 ± 0.07 (105 %)
right	0.37 ± 0.03	0.30 ± 0.04 (81 %)*	0.35 ± 0.10 (94 %)	0.38 ± 0.07 (103 %)
Medial temporal cortex	0.20 ± 0.02	0.17 ± 0.03 (83 %)*	0.19 ± 0.02 (93 %)	0.20 ± 0.03 (100 %)
Caudate	0.36 ± 0.03	0.24 ± 0.10 (68 %)*	0.31 ± 0.06 (86 %)	0.34 ± 0.07 (95 %)
left	0.36 ± 0.03	0.22 ± 0.11 (62 %)*	0.31 ± 0.06 (86 %)	0.35 ± 0.05 (98 %)
right	0.36 ± 0.04	0.27 ± 0.09 (75 %)*	0.31 ± 0.06 (87 %)	0.33 ± 0.10 (93 %)
Putamen	0.40 ± 0.03	0.32 ± 0.06 (80 %)*	0.35 ± 0.06 (87 %)*†	0.42 ± 0.08 (103 %)
Thalamus	0.36 ± 0.03	0.29 ± 0.06 (80 %)*	0.31 ± 0.06 (85 %)*	0.35 ± 0.06 (97 %)
Cerebellar cortex	0.29 ± 0.02	0.27 ± 0.05 (92 %)	0.27 ± 0.06 (92 %)†	0.31 ± 0.05 (107 %)
Orbital frontal cortex[O]	0.34 ± 0.03	0.28 ± 0.07 (82 %)*	0.29 ± 0.06 (87 %)	0.34 ± 0.06 (99 %)
left	0.34 ± 0.03	0.27 ± 0.07 (81 %)*	0.30 ± 0.06 (87 %)	0.33 ± 0.08 (98 %)
right	0.34 ± 0.03	0.28 ± 0.08 (82 %)*	0.29 ± 0.05 (86 %)	0.34 ± 0.04 (101 %)
Parieto-frontal cortex[O]	0.30 ± 0.02	0.26 ± 0.05 (87 %)*	0.27 ± 0.04 (89 %)	0.30 ± 0.05 (98 %)
left	0.29 ± 0.02	0.26 ± 0.05 (87 %)*	0.27 ± 0.05 (91 %)	0.28 ± 0.06 (96 %)
right	0.31 ± 0.02	0.27 ± 0.06 (87 %)*	0.27 ± 0.04 (87 %)	0.31 ± 0.04 (100 %)

* Significantly different from controls, $p < 0.05$
† Significantly different from the non-demented DZ co-twins in post-hoc tests using Bonferroni adjustment for multiple comparisons, corrected $p < 0.05$
[O] Placement of ROIs guided by SPM results between the non-demented MZ co-twins and controls

5.2 Midlife risk factor analyses (studies III and IV)

5.2.1 Study population characteristics

A total of 2 926 subjects for whom at least some questionnaire data were available were contacted for TELE, but 26 of them were dead at the time of the attempted interview (i.e. they had died since the latest update at the Population Register Centre for vital status of the cohort). This left 2 900 contactable subjects, but 288 of those had missing information on educational level and were excluded. Additionally, 447 subjects with

adequate data were not interviewed fully (72 not reached, 282 declined/proxy declined, 93 not interviewed fully for other reasons). This left 2 165 fully interviewed subjects with adequate questionnaire data to be included in the analyses (see **Figure 4**). The dead subjects would have been older at the time of the interview than the contacted subjects, but did not differ in terms of sex, educational level, or any of the assessed risk factors. The 735 subjects not included in the analyses were older, more often women and less educated than the included subjects. When controlling for age, sex and educational level, those not included were not more likely to have high blood pressure or cholesterol level, to be obese, to be physically sedentary or to report heavy drinking or binge drinking, but more often reported pass-outs due to drinking.
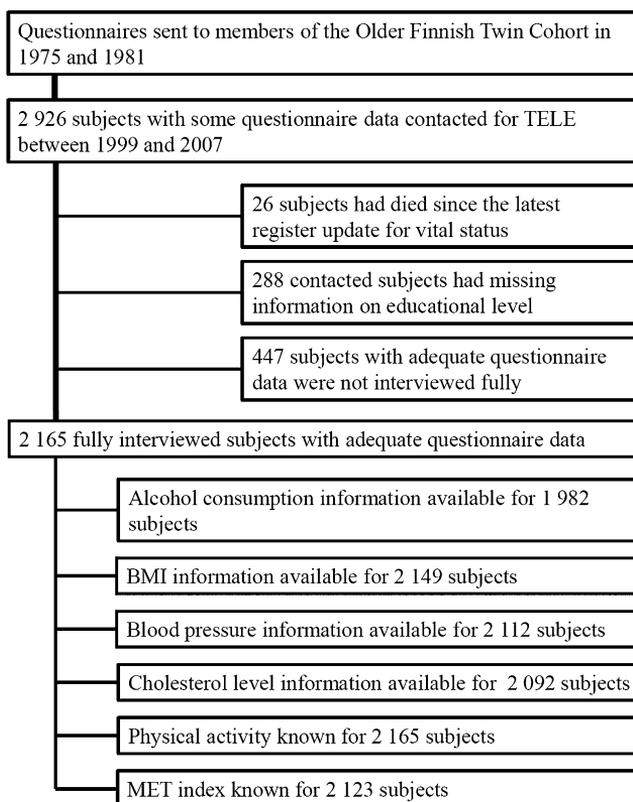

Figure 4. Flowchart of the study design in studies **III** and **IV**.

At baseline in 1981, the successfully interviewed subjects with questionnaire data were 51.7 ± 6.1 years old (mean ± SD), and at the time of the interviews they were 74.3 ± 5.2 years old. Follow-up time was 22.6 ± 2.3 years, and 51.1 % of the subjects were men. Most, 59.6 %, were classified as cognitively intact, 25.2 % as having MICF and 15.2 % as cognitively impaired. The cognitively impaired subjects were older, less educated, and more likely to have at least one ε4 allele. They were also more likely to be non-drinkers, report more than two pass-outs in 1981, be obese, have hypertension or hypercholesterolemia, and be physically inactive. Detailed information on the subjects is

provided in Tables 1 and 2 of Original Publication III and in Table 2 of Original Publication IV.

In the alcohol consumption analyses only subjects with adequate information on all consumption variables (total consumption in 1981, binge drinking in 1975 and 1981, number of pass-outs in 1981) were included and additionally one subject reporting daily consumption of over 200 grams of pure ethanol was excluded, leaving 1 982 subjects. In the cardiovascular risk factor analyses, the number of subjects with baseline data varied between different risk factors, and the number of subjects in each analysis is shown in **Figure 4** and together with the results.

5.2.2 Midlife alcohol consumption and risk of cognitive impairment (study III)

In the multinomial regression analysis, non-drinkers and heavy drinkers had an increased risk of cognitive impairment compared to light drinkers (RR 1.46, 95 % CI 1.02-2.10 and RR 1.94, 95 % CI 1.10-3.44, respectively) when controlling for age, sex and educational level. In the unadjusted model, heavy drinkers did not have a significantly increased risk for cognitive impairment, and the risk associated with non-drinking was somewhat higher than in the adjusted model. All RRs are shown in **Table 6**. Since there was no significant interaction between alcohol consumption and sex, educational level or smoking status on cognitive impairment risk, subgroup analyses are not presented.

In order to include only lifelong non-drinkers the analyses of Model 1 were repeated excluding the 155 individuals who either reported being non-drinkers in 1981 but not in 1975, or were non-drinkers but in 1975 reported having drunk more during some phase of their life. The results did not differ from the main analyses, as non-drinkers had an RR of 1.87 (95 % CI 1.25-2.82) in this analysis. Additionally, stopping drinking between 1975 and 1981 was not associated with an increased cognitive impairment risk compared to continual drinking (RR 0.78, 95 % CI 0.45-1.35).

The association between alcohol consumption and cognitive impairment was not affected by controlling for changes in drinking over time, as the results of Model 1 were similar when estimating drinking using the mean of consumption in 1975 and 1981 (RR 1.84, 95 % CI 1.25-2.72 for non-drinkers and RR 2.20, 95 % CI 1.25-3.86 for heavy drinkers). In an analysis including only subjects whose drinking category remained stable from 1975 to 1981 non-drinkers had a significantly increased risk of cognitive impairment (RR 2.10, 95 % CI 1.36-3.24). Heavy drinkers did not have any significantly elevated risk, but this group included only 59 subjects.

Table 6. RRs and 95 % CIs for cognitive impairment associated with midlife alcohol consumption. In the binge drinking and pass-out analyses, non-drinkers were excluded.

	Unadjusted model		Model 1*		Model 2[O]	
	RR	95 % CI	RR	95 % CI	RR	95 % CI
Alcohol consumption in 1981	(n = 1982)		(n = 1982)		(n = 1 486)	
Non-drinker	1.92	(1.39-2.65)[†]	1.46	(1.02-2.10)[†]	1.41	(0.90-2.20)
Light drinker	1.00		1.00		1.00	
Moderate drinker	0.88	(0.62-1.25)	0.95	(0.63-1.42)	0.92	(0.57-1.47)
Heavy drinker	1.40	(0.84-2.33)	1.94	(1.10-3.44)[†]	2.64	(1.40-4.95)[†]
Binge drinking in 1975 and 1981	(n = 1 531)		(n = 1 531)		(n = 1161)	
Neither 1975 nor 1981	1.00		1.00		1.00	
Only 1975 or 1981	1.58	(0.98-2.55)	1.79	(1.00-3.19)	1.80	(0.87-3.71)
Both 1975 and 1981	1.56	(1.01-2.41)[†]	1.98	(1.08-3.64)[†]	1.65	(0.79-3.46)
Number of pass-outs in 1981	(n = 1 531)		(n = 1 531)		(n = 1161)	
0	1.00		1.00		1.00	
1-2	1.22	(0.72-2.08)	1.00	(0.54-1.87)	0.98	(0.49-1.98)
>2	4.92	(2.14-11.3)[†]	3.85	(1.51-9.83)[†]	3.82	(1.11-13.1)[†]

* Analyses controlled for age, sex and educational level. In binge drinking and pass-out analyses, also total consumption was controlled.
[O] Analyses controlled for age, sex, educational level and number of ε4 alleles. In binge drinking and pass-out analyses, also total consumption was controlled.
[†] Significant risk increase, p < 0.05

In addition to the effects of total alcohol consumption, the effects of different drinking patterns were analyzed. Non-drinkers were excluded from these analyses, leaving 1 531 subjects. Binge drinking in 1975 and 1981 as well as at least three pass-outs in 1981 were risk factors for cognitive impairment in the unadjusted model. In the multivariate analyses, also total consumption was controlled for. In Model 1, at least monthly binge drinking in only 1975 or 1981 was a borderline significant risk factor for cognitive impairment (RR 1.79, 95 % CI 1.00-3.19, and binge drinking in both 1975 and 1981 significantly increased the risk of cognitive impairment (1.98, 95 % CI 1.08-3.64). In addition, at least three reported pass-outs in 1981 were a risk factor for cognitive impairment (RR 3.85, 95 % CI 1.51-9.83). The RRs are shown in **Table 6**.

In Model 2, which adjusted also for the number of ε4 alleles, heavy drinking and at least three pass-outs in 1981 were risk factors for cognitive impairment (RR 2.64, 95 % CI 1.40-4.95 and RR 3.82, 95 % CI 1.11-13.12, respectively), whereas non-drinking was not (RR 1.41, 95 % CI 0.90-2.20). Monthly binge drinking was not a significant risk factor (RR 1.65, 95 % CI 0.79-3.46 for monthly binge drinking in 1975 and 1981). The results are shown in **Table 6**. There is some evidence that the effects of drinking on dementia risk might interact with ApoE genotype (Anttila et al. 2004; Luchsinger et al. 2004; Ruitenberg et al. 2002). In study **III**, the interaction between ApoE genotype and number of pass-outs significantly affected the associated cognitive impairment risk (p<0.01), and also the interaction between binge drinking and ApoE genotype was borderline significant (p=0.06). Therefore, the results for Model 1 are presented also separately for ApoE ε4 carriers and non-carriers. The results for non-carriers were

similar to the main analyses including all subjects, but no significant associations were found between drinking and cognitive impairment risk in ε4 carriers. The results are shown in **Table 7**.

Table 7. RRs with 95 % CIs for cognitive impairment separately for ApoE ε4 carriers and non-carriers adjusted for age, sex and educational level. In binge drinking and pass-out analyses, non-drinkers were excluded.

	ε4 non-carriers		ε4 carriers	
	RR	**95 % CI**	**RR**	**95 % CI**
Alcohol consumption in 1981	**(n = 1 005)**		**(n = 481)**	
Non-drinker	1.77	(1.03-3.05)[†]	0.94	(0.41-2.14)
Light drinker	1.00		1.00	
Moderate drinker	0.75	(0.40-1.41)	1.18	(0.56-2.46)
Heavy drinker	3.02	(1.36-6.69)[†]	2.02	(0.72-5.64)
Binge drinking in 1975 and 1981	**(n = 790)**		**(n = 371)**	
Neither 1975 nor 1981	1.00		1.00	
Only 1975 or 1981	2.59	(1.05-6.35)[†]	1.05	(0.35-3.12)
Both 1975 and 1981	2.77	(1.01-7.58)[†]	0.80	(0.26-2.40)
Number of pass-outs in 1981	**(n = 790)**		**(n = 371)**	
0	1.00		1.00	
1-2	1.55	(0.60-3.97)	0.48	(0.18-1.30)
>2	11.23	(2.67-47.1)[†]	0.35	(0.02-5.47)

[†] Significant risk increase, p < 0.05

Two additional models were tested. In a model in which total alcohol consumption in 1981, binge drinking in 1975 and 1981, and pass-outs in 1981 were included while controlling for age, sex, educational level, and smoking status in 1981, non-drinkers had a significantly increased risk for cognitive impairment (RR 1.47, 95 % CI 1.02–2.13). In contrast, heavy drinking was not associated with any increased cognitive impairment risk (RR 1.13, 95 % CI 0.56-2.30). Two or more pass-outs in 1981 were a risk factor for cognitive impairment (RR 2.78, 95 % CI 1.07–7.23). No significant increase in cognitive impairment risk was found in subjects who reported binge drinking either in 1975 or 1981 or both (RR 1.51, 95 % CI 0.83-2.74 and RR 1.64, 95 % CI 0.88-3.05, respectively). In a model controlling for age, sex, educational level, blood pressure level, cholesterol level and BMI, the risk associated with heavy drinking and at least two pass-outs in 1981 remained significant (RR 1.83, 95 % CI 1.03-3.26 and RR 2.86, 95 % CI 1.07–7.63, respectively).

To test whether the association between alcohol consumption and cognitive impairment was independent of familial or genetic factors co-twin control analyses including twin pairs clearly discordant for cognitive function were done. These analyses included 46-55 twin pairs, and none of the ORs were statistically significant. Non-drinkers and heavy drinkers had ORs of 0.90 (95 % CI 0.32-2.49) and 1.47 (95 % CI 0.24-8.89) compared to light to moderate drinkers, respectively. When controlling for total consumption, monthly binge drinking in 1975 and/or 1981 had an OR of 1.95 (95 % CI 0.26-14.5), and passing out at least once in 1981 an OR of 3.73 (95 % CI 0.36-40.3). Power estimates to detect a doubled risk of cognitive impairment were 14.1-40.9 %.

5.2.3 Midlife cardiovascular risk factors and risk of cognitive impairment (study IV)

In the unadjusted model, obesity, high blood pressure and cholesterol levels, and low leisure time physical activity were significant risk factors for cognitive impairment. The risk estimates were slightly attenuated when controlling for age, sex and educational level. In Model 1, midlife obesity was a significant risk factor for cognitive impairment in later life (RR 2.42, 95 % CI 1.47-3.99). In addition, hypertension (RR 1.38, 95 % CI 1.01-1.87), physical inactivity (RR 2.49, 95 % CI 1.10-5.67) and belonging to the first MET index quartile (RR 1.86, 95 % CI 1.26-2.72) were significant risk factors, whereas a high cholesterol level was not associated with any significantly elevated risk. The results were unaffected by excluding those subjects with unknown blood pressure or cholesterol levels. In Model 2, which controlled also for the number of ε4 alleles, only belonging to the first MET index quartile remained a significant risk factor for cognitive impairment (RR 1.75, 95 % CI 1.11-2.75), and the risk associated with hypertension was borderline significant. The risks associated with obesity and being physically sedentary were clearly attenuated. The RRs for all models are shown in **Table 8**. Interactions between any of the assessed risk factors and sex or educational level did not significantly contribute to the cognitive impairment risk. Thus, no subgroup results are presented.

Table 8. RRs and 95 % CIs for cognitive impairment associated with midlife cardiovascular risk factors.

	Unadjusted model		Model 1*		Model 2[O]	
	RRR	95 % CI	RRR	95 % CI	RRR	95 % CI
BMI	(n = 2 149)		(n = 2 149)		(n = 1 601)	
Lean or normal	1.00		1.00		1.00	
Overweight	1.21	(0.92-1.59)	1.02	(0.76-1.35)	0.88	(0.63-1.23)
Obese	2.77	(1.75-4.37)[†]	2.42	(1.47-3.99)[†]	1.23	(0.66-2.29)
Blood pressure	(n = 2 112)		(n = 2 112)		(n = 1 574)	
Normal	1.00		1.00		1.00	
Not measured	1.41	(0.92-2.15)	1.49	(0.96-2.31)	1.21	(0.68-2.14)
High	1.44	(1.08-1.91)[†]	1.38	(1.01-1.87)[†]	1.38	(0.97-1.97)
Cholesterol level	(n = 2 092)		(n = 2 092)		(n = 1 563)	
Normal	1.00		1.00		1.00	
Not measured	1.10	(0.84-1.44)	1.12	(0.84-1.50)	1.25	(0.88-1.77)
High	1.78	(1.11-2.84)[†]	1.51	(0.91-2.50)	1.63	(0.91-2.90)
Physical activity	(n = 2 165)		(n = 2 165)		(n = 1 611)	
Conditioner	1.00		1.00		1.00	
Occasional exerciser	2.74	(1.41-5.29)[†]	1.92	(0.91-4.05)	1.13	(0.53-2.40)
Sedentary	4.06	(1.94-8.47)[†]	2.49	(1.10-5.67)[†]	1.53	(0.66-3.56)
Leisure time MET quartiles	(n = 2 123)		(n = 2 123)		(n = 1 581)	
IV	1.00				1.00	
III	1.10	(0.76-1.61)	1.14	(0.77-1.69)	1.10	(0.69-1.75)
II	1.64	(1.15-2.33)[†]	1.33	(0.91-1.93)	1.17	(0.75-1.82)
I	2.63	(1.85-3.74)[†]	1.86	(1.26-2.72)[†]	1.75	(1.11-2.75)[†]

* Analyses controlled for age, sex and educational level
[O] Analyses controlled for age, sex, educational level and number of apolipoprotein E ε4 alleles
[†] Significant risk increase, p < 0.05

Cross sectional analyses of the Framingham Offspring Cohort have suggested that the effects of a combination of cardiovascular risk factors and also of obesity on neuropsychological performance are modified by ApoE genotype, so that the associations are stronger in ε4 carriers (Zade et al. 2010; Zade et al. 2011). Therefore, even though the interactions between any of the assessed risk factors and ApoE genotype did not significantly affect the associations (p>0.15), subgroup analyses based on ApoE genotype are shown in **Table 9**. In these analyses, obesity was not associated with an increased risk of cognitive impairment in either subgroup. Hypertension remained a borderline significant risk factor only in individuals with at least one ε4 allele. Hypercholesterolemia or being classified as physically sedentary did not increase the risk of cognitive impairment in either subgroup, but the RR point estimates were higher in the ε4 carriers. Finally, belonging to the first MET index quartile increased the risk of cognitive impairment only among the ε4 carriers as compared to the fourth quartile (RR 2.83, 95 % CI 1.34-5.99).

Table 9. RRs with 95 % CIs for cognitive impairment separately for ApoE ε4 carriers and non-carriers adjusted for age, sex and educational level.

	ε4 non-carriers		ε4 carriers	
	RR	95 % CI	RR	95 % CI
BMI	(n = 1087)		(n = 514)	
Lean or normal	1.00		1.00	
Overweight	0.93	(0.62-1.41)	0.79	(0.46-1.37)
Obese	1.29	(0.52-3.22)	1.16	(0.49-2.75)
Blood pressure	(n = 1065)		(n = 509)	
Normal	1.00		1.00	
Not measured	1.19	(0.52-2.72)	1.25	(0.58-2.73)
High	1.19	(0.76-1.88)	1.75	(0.96-3.17)
Cholesterol level	(n = 1059)		(n = 504)	
Normal	1.00		1.00	
Not measured	1.10	(0.71-1.71)	1.47	(0.82-2.62)
High	1.35	(0.62-2.94)	2.00	(0.82-4.87)
Physical activity	(n = 1094)		(n = 517)	
Conditioner	1.00		1.00	
Occasional exerciser	1.19	(0.48-2.92)	0.99	(0.25-3.96)
Sedentary	1.32	(0.47-3.68)	2.01	(0.45-8.91)
Leisure time MET quartiles	(n = 1075)		(n = 506)	
IV	1.00		1.00	
III	1.06	(0.60-1.88)	1.12	(0.50-2.48)
II	1.11	(0.64-1.94)	1.21	(0.58-2.53)
I	1.36	(0.77-2.41)	2.83	(1.34-5.99)*

* Significant risk increase, p < 0.05

Part of the subjects born in 1930 and after also received questionnaires in 1990. Using these data, the effects of longstanding hypertension or hypercholesterolemia were studied. These analyses examined 794 subjects for hypertension and 285 subjects for hypercholesterolemia. Subjects with reported hypertension in both 1981 and 1990 had an increased risk for cognitive impairment compared to subjects with normal blood pressure levels in 1981 and 1990 (RR 2.00, 95 % CI 1.10-3.59), whereas no risk increase

was found for subjects with hypertension only in 1990 (RR 0.62, 95 % CI 0.25-1.54). Similar analyses for hypercholesterolemia showed no significant risk increases (RR 1.49, 95 % CI 0.34-6.51 for subjects with hypercholesterolemia in 1981 and 1990). In addition, subjects who were overweight in 1981 were studied in detail (n = 408). Among them those who gained more than 10 % in weight by 1990 had an increased risk of cognitive impairment as compared to subjects whose weight did not change by more than 10 % in either direction (RR 4.27, 95 % CI 1.62-11.2). Only 15 overweight subjects had lost more than 10 % of their weight by 1990.

A full model including BMI, blood pressure level, cholesterol level and MET index quartile controlling for age, sex and educational level was also evaluated. Obesity and belonging to the first MET index quartile remained significant risk factors for cognitive impairment (RR 2.02, 95 % CI 1.19-4.43 and RR 1.74, 95 % CI 1.16-2.60, respectively), whereas hypercholesterolemia or hypertension were not associated with cognitive impairment risk (RR 1.26, 95 % CI 0.91-1.74 and 1.57, 95 % CI 0.93-2.64, respectively). When also controlling for the number of ε4 alleles, only belonging the first MET index quartile and hypercholesterolemia increased cognitive impairment risk (RR 1.63, 95 % CI 1.00-2.65 and RR 1.89, 95 % CI 1.04-3.42, respectively). Furthermore, the risk associated with obesity was clearly attenuated when controlling for ApoE status (RR 1.05, 95 % CI 0.53-2.09).

There is some evidence that the protective effects of physical exercise on the central nervous system might not be completely vascular (Dishman et al. 2006). In order to test the effects of physical inactivity independent of other cardiovascular factors, a model controlling for age, sex, educational level and the number of other cardiovascular risk factors (i.e. obesity, hypertension, hypercholesterolemia) was tested. In this model belonging to the first MET index quartile was a borderline significant risk factor for cognitive impairment as compared to the fourth quartile (RR 1.85, 95 % CI 0.97-3.51). In contrast, the risk of the physically sedentary subjects was not increased as compared to the conditioners (RR 1.57, 95 % CI 0.38-6.51).

In co-twin control analyses involving those twin pairs clearly discordant for cognitive function, the number of included twin pairs was 54-67, and the estimated statistical power to detect an OR of 1.5 was 8.2-17.1 %. No significant risk increases were found, but the OR point estimates were mostly in line with RRs of the main analyses: 1.25 (95 % CI 0.49-3.17) for hypertension, 1.50 (95 % CI 0.42-5.32) for hypercholesterolemia, 2.00 (95 % CI 0.60-6.64) for physical inactivity and 2.33 (95 % CI 0.90-6.07) for the fourth MET index quartile. In contrast, the OR associated with obesity was clearly attenuated (OR 1.33, 95 % CI 0.30-5.96)

5.2.4 Dementia risk score (study IV)

Subjects with unknown blood pressure or cholesterol level were excluded from the risk score analyses, as were subjects with missing information on one or more of the examined risk factors and those classified as having MICF. This left a total of 591 subjects, for 439 of whom also ApoE genotype was known. The subjects included in the risk score analyses did not differ from the cognitively intact or impaired subjects not

included in terms of age, educational level, or in the prevalence of obesity, hypercholesterolemia or high leisure time physical activity. However, they were more often men, and more likely to have at least one ε4 allele or hypertension. The subjects were 51.8 ± 6.2 years old in 1981 and 74.4 ± 5.3 years at the time of the interview. Furthermore, 57.0 % of the subjects were men.

The dementia risk score including age, sex, educational level, blood pressure, BMI, cholesterol level and physical inactivity had an AUC value of 0.742 (95 % CI 0.694-0.789), whereas the score including also the number of ε4 alleles had an AUC of 0.754 (95 % CI 0.696-0.812). The difference between the models was non-significant (p=0.24). The results of the ROC analyses are shown in **Figure 5**.
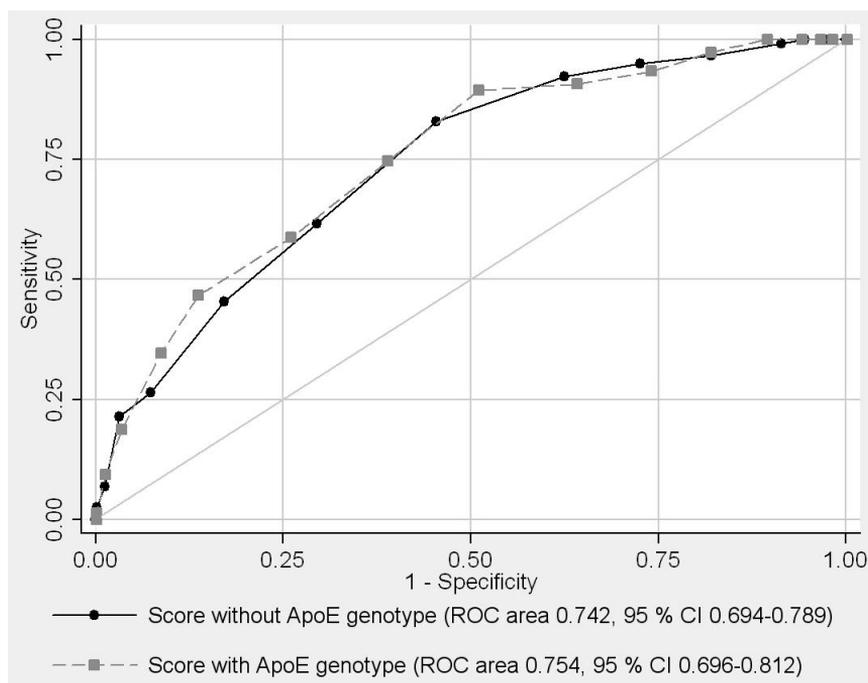
Figure 5. Results for the ROC analyses of the two dementia risk scores. The figure has been published in Original Publication IV.

Comparing the risk score quartiles revealed a strong association between the risk score and the risk of cognitive impairment. In the score without ApoE status, subjects in the highest quartile had a RR of 14.2 (95 % CI 5.79-34.7) as compared to those in the lowest quartile. The corresponding RR for the score including ApoE status was 8.89 (95 % CI 3.38-23.4). All RRs are shown in **Figure 6**. When the risk scores were treated as continuous variables, there was a gradual increase in the predicted probability of cognitive impairment with increasing scores. A graphical illustration of the predicted probabilities with 95 % CIs is shown in **Figure 7**.
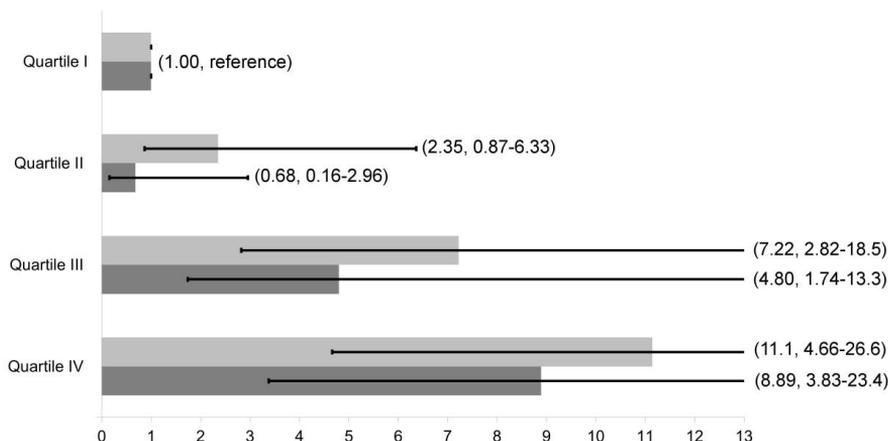
Figure 6. Illustration of RRs (bars) and 95 % CIs (whiskers) for dementia risk score quartiles. RRs and 95 % CIs are shown in brackets. Lighter bars indicate the score without ApoE status, darker bars the score with ApoE status.
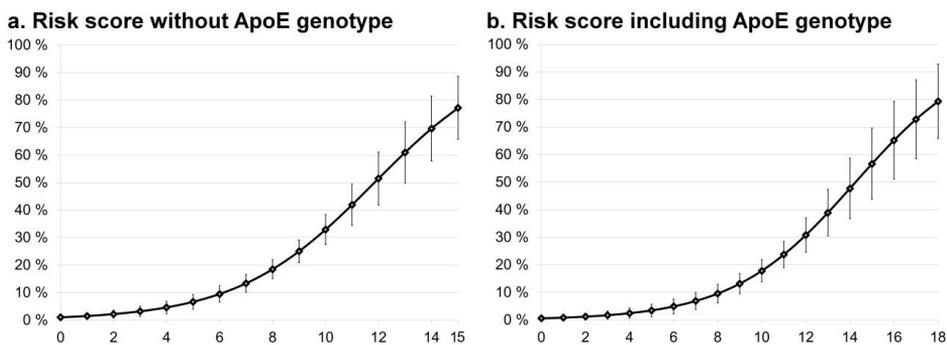

Figure 7. Illustration of predicted probabilities of cognitive impairment with 95 % CIs (whiskers) for both risk scores. a) Risk score without ApoE genotype b) Risk score including ApoE genotype

To further evaluate the accumulation of multiple risk factors, a logistic regression analysis was done comparing subjects without any of the assessed risk factors to the subjects with one to four of the risk factors. The exclusion criteria in this analysis were similar to the risk score analyses. As the analysis was controlled for age, sex and educational level, it reflects the impact of vascular factors alone. Because only one subject had all the assessed risk factors, subjects with three to four risk factors were pooled together. The accumulation of risk factors clearly increased an individual's risk of cognitive impairment, as subjects with three to four risk factors had a fourfold risk of cognitive impairment when compared to subjects with no risk factors (OR 3.95, 95 % CI 1.15-13.6). The cognitive impairment risk of those with 3-4 risk factors was also significantly higher as compared to those with only one risk factor (p=0.04). All the ORs are shown in **Figure 8**.
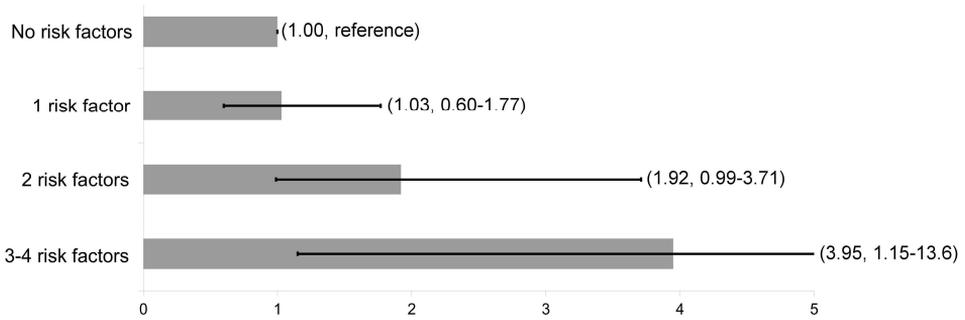
Figure 8. ORs (bars) with 95 % CIs (whiskers) for cognitive impairment associated with multiple risk factors. Subjects without risk factors were used as the reference. Numeric values of ORs and 95 % CIs are shown in brackets.

6. DISCUSSION

6.1 Cerebral glucose metabolic rate in twins discordant for Alzheimer's disease (studies I and II)

6.1.1 Genetically mediated hypometabolism in Alzheimer's disease

The demented DZ co-twins had extensively reduced rGMR compared to the controls in the manual ROI analysis of study **I**. In line with previous findings in AD patients (Herholz 2003; Herholz et al. 2007), rGMR was relatively spared in the primary sensory, motor and visual cortices as well as in the cerebellar cortex. In contrast to the demented co-twins, no differences were found between the non-demented DZ co-twins and controls. Similar results were seen when analyzing rGMR values relative to sensory motor cortex. In a previous FDG-PET study using similar methodology on MZ twin pairs discordant for AD, also the non-demented co-twins showed reduced rGMR in lateral temporal and parietal cortices (Järvenpää et al. 2003).

The lack of significant differences between the controls and non-demented DZ co-twins in study **I** could have resulted from methodological limitations, as ROI based analysis of rGMR does not assess the whole brain space for possible differences, and requires assumptions on the topography of possible differences. Similarly, possible differences between controls and non-demented co-twins outside the defined ROIs could have been missed in the previous study on MZ twins. Manually determining the ROIs for each subject is also prone to operator induced bias.

To overcome these shortcomings, an SPM analysis of both MZ and DZ twin pairs discordant for AD was conducted in study **II**. This enabled voxel-level analysis of the whole brain space without any *a priori* hypothesis on the topography of possible differences between study groups. In the SPM analysis, the demented co-twins showed extensively reduced GMR compared to controls, and the automated ROI analysis revealed that the cortical reductions were greatest in posterior cingulate, medial temporal and parietal cortex. rGMR was also greatly reduced in subcortical grey matter structures. The non-demented MZ co-twins showed reduced rGMR in inferior frontal, lateral temporal, parietal and medial temporal cortex, as well as in thalamus, putamen and right amygdala. Automated ROI analysis showed the reductions to be greatest in subcortical grey matter structures, parietal cortex and temporal cortex. In contrast, no reductions were seen in the non-demented DZ co-twins even when the whole brain space was analyzed. Additionally, a correlation between genetic load and rGMR was seen in lateral temporal cortex.

The SPM comparison between the non-demented MZ and DZ co-twins revealed similar findings to the comparison of controls and non-demented MZ co-twins. The post-hoc comparisons of the automated ROI analyses showed that the non-demented MZ co-twins had significantly lower rGMR in parietal cortex, putamen and cerebellar cortex than the

non-demented DZ co-twins, but the difference in cerebellar cortex is most likely artefactual. Taken together, these direct comparisons between the non-demented MZ and DZ co-twin add to the evidence that the GMR of the non-demented DZ did not differ from that of the controls.

Together, studies **I** and **II** represent the largest FDG-PET study on twins discordant for AD, as all previous studies except for the study by Järvenpää et al. (2003) have included only one to three twin pairs (Kumar et al. 1991; Luxenberg et al. 1987; Small et al. 1993). The current study is also the first to include both MZ and DZ twin pairs discordant for AD, and therefore makes it feasible to evaluate the contributions of genetic and shared environmental factors to AD susceptibility and cerebral GMR. Whereas MZ co-twins share all their genes, DZ co-twins are genetically full siblings sharing on average half of their segregating genes. Both MZ and DZ twin pairs are assumed to share environmental factors equally. Hence, the different findings in the non-demented MZ and DZ co-twins can be attributed to differences in their genetic characteristics. Additionally, the underlying genetic factors do not seem strong enough to differentiate non-demented DZ co-twins from unrelated controls. This is somewhat expected, as concordance studies have clearly shown that both genetic and environmental factors contribute to the pathogenesis of sporadic AD (Raiha et al. 1996; Bergem et al. 1997; Gatz et al. 2006). In a purely additive genetic model, one would expect to see a continuum from the controls through the non-demented DZ co-twins to the non-demented MZ co-twins. In study **II**, no evidence of such a continuum was seen, as no difference between the controls and non-demented DZ co-twin was seen. Therefore, it seems likely that a combined effect of several genes is involved in the genetic susceptibility to the physiological changes instead of a purely additive effect of individual genes. This is because DZ co-twins would be less likely to share exactly the same combination of alleles since genes segregate independently at each locus. The present sample size is naturally too small to conclusively distinguish between these two genetic models.

It is possible that the reduced rGMR in the non-demented MZ co-twins is a manifestation of an early phase of symptomatic AD. This is unlikely because the non-demented MZ and DZ co-twins did not differ significantly in their MMSE scores (difference only 0.3 points), but definite exclusion of this possibility would require follow-up of the twin pairs. It is more likely that the reductions are an indication of genetic susceptibility to AD, or of genetically determined mechanisms in AD pathogenesis. This is supported by the recent PIB-PET study on Finnish twin pairs discordant for AD showing increased PIB uptake in the non-demented MZ co-twins compared controls in temporal cortex, parietal cortex, posterior cingulate cortex and putamen. These regions are typically affected by Aβ pathology in AD. In contrast, in the non-demented DZ co-twins, no increased PIB uptake was detected even when the whole brain space was analyzed. (Scheinin et al. 2011) A similar MRI study on discordant MZ twin pairs observed no significant differences between controls and non-demented MZ co-twins in hippocampal volume (Järvenpää et al. 2004), suggesting that functional PET

imaging is able to detect the hypothesized genetic susceptibility even when no structural changes are seen.

In early AD, rGMR reductions are seen first in posterior cingulate cortex followed by posterior parts of temporal and parietal cortices (Minoshima et al. 1997). In study **II**, the non-demented MZ co-twins had reduced rGMR in temporal and parietal cortex, but not in posterior cingulate cortex. Additionally, rGMR was reduced in subcortical grey matter structures, and overall the absolute rGMR values seemed lower than the corresponding values of the controls. No differences were found between the non-demented co-twins and controls in relative rGMR. Therefore, it is plausible that the non-demented MZ co-twins exhibit genetically determined background hypometabolism, possibly related to vulnerability to AD. The previous ROI study of the MZ twin pairs possibly failed to detect this because only limited parts of brain space were analyzed. However, it has been shown that AD patients with early disease onset (before the age of 65) have more extensive reductions in GMR as compared to patients with later disease onset, and the topography of reduced GMR in early-onset patients has been suggested to be similar to that seen in the non-demented MZ co-twins of study **II**, including also reductions in subcortical structures (Kim et al. 2005).

It is also possible that the reduced rGMR values seen in the non-demented MZ co-twins reflect some heritability of cerebral GMR, a phenomenon which has been studied in very little detail. Only one study of seven MZ female twin pairs has assessed the similarity of rGMR in healthy co-twins (Clark et al. 1988). Significant intraclass correlations were found between the rGMR values of the co-twins in prefrontal cortex, orbitofrontal cortex and basal ganglia structures (caudate and putamen). Further statistical analysis suggested that the significant correlations in these regions were not independent but instead reflected the same metabolic phenomena. The small sample size, lack of dizygotic pairs and inclusion of only female twins limit the generalization of these findings. It has been shown that cognitively intact ApoE ε4 carriers have reduced rGMR in regions affected by AD (Small et al. 2000; Reiman et al. 2004; Reiman et al. 2005). Unfortunately, the number of subjects in studies **I** and **II** was too small to clarify whether the reduced rGMR seen in non-demented MZ co-twins could be attributed to the ε4 allele.

6.1.2 Methodological considerations in studies I and II

Some caution must be used in the interpretation of the results of studies **I** and **II**. Firstly, the number of examined twin pairs was small. However, it is unlikely that significant reductions in non-demented DZ co-twins were not detected, since their rGMR values were practically identical to those of the controls. The small number of subjects rather reflects the rarity of suitable twin pairs, as hundreds of twin pairs were screened for studies **I** and **II**. The MZ twin pairs consisted entirely of women, and even though the results were unaffected by the exclusion of male subjects from all groups, it is possible that the results cannot be generalized to both sexes. Finally, one of the non-demented MZ co-twins was suspected to have MCI, but the differences between controls and non-demented MZ co-twins remained significant even when this twin pair was excluded.

Partial volume correction was not done in studies **I** and **II**, which might have affected the results, especially in the demented co-twins. Partial volume effects are attributable to the limited spatial resolution of PET scanners, and they confound the assessment of tracer concentration in small structures and areas where grey matter is in close proximity to CSF. This could have affected the assessment of rGMR, especially in hippocampus and other medial temporal lobe structures (Samuraki et al. 2007). The lack of partial volume correction could also partly explain the findings of markedly reduced rGMR values in subcortical grey matter structures in both non-demented MZ and demented co-twins (Frouin et al. 2002). In the demented co-twins, also displacement of subcortical structures during normalization to a common stereotactic space due to ventricular enlargement might have affected the results, even though also the manual ROI analysis of the demented DZ co-twins estimated an almost equally severe reduction of rGMR in these structures. There is also evidence that spatial normalization causes mismatches at ventricular surfaces, which could have affected the results of the SPM and automated ROI analyses in thalamus and caudate (Ishii et al. 2001).

There were some discrepancies between the voxel-level SPM and automated ROI analyses. In SPM analysis, the cluster peak in the comparison between the controls and non-demented MZ co-twins was located in cerebellum, and a significant difference was observed in orbitofrontal cortex, but in the ROI analyses, no differences between the two groups were seen in either region. This could be a result of the different statistical methods used, as ANOVA does not control for the twin structure of the data. However, because both areas are located in the inferior parts of the brain, it is possible that the results of the SPM analysis in this area are partly artefactual.

Finally, the statistical methods used in study **I** were not entirely suitable for the twin design, as one-way ANOVA does not take possible correlations between co-twins into account. However, the main focus in the analyses was on the difference between the independent groups of non-demented co-twins and controls, and the differences between these groups were clearly non-significant.

6.2 Midlife risk factors for later cognitive impairment (studies III and IV)

6.2.1 Midlife alcohol consumption and risk of cognitive impairment (study III)

In study **III**, an increased risk of cognitive impairment was associated with increasing total consumption of alcohol when adjusting for confounders. Non-drinkers were also at an increased risk compared to light drinkers. In addition, study **III** was one of the first to report an increased risk of cognitive impairment associated with consuming large amounts of alcohol at one and the same occasion. This effect was seen even when controlling for total alcohol consumption, suggesting that this so-called binge drinking is an independent risk factor for cognitive impairment. In an unadjusted model, heavy drinking was not associated with significantly increased risk of cognitive impairment,

and the risk associated with abstaining was higher than in the adjusted model. This suggests that drinking habits are not independent of sex, age and educational level.

It has been proposed that the estimation of alcohol consumption only at baseline may lead to an underestimation of the associated risks particularly with heavy drinking (Emberson et al. 2005). To take this into consideration, the analyses were repeated using the mean of consumption in 1975 and 1981, and also including only subjects with stable drinking behavior during the six years, but the results did not differ from the main analyses. There is evidence that alcohol consumption tends to decline with increasing age, and that this decrease is associated with or caused by a development of ill health, perhaps even leading to total cessation of drinking (Wannamethee and Shaper 1998). As the results of study **III** regarding non-drinkers were not affected by excluding former drinkers, and drinking cessation between 1975 and 1981 did not increase cognitive impairment risk, this so called sick quitter effect probably did not affect the findings. However, reported lifetime abstinence has been shown to be prone to error even when using multiple measurements of drinking (Rehm et al. 2008), and non-drinkers differ from light drinkers in characteristics not controlled for in study **III** (Fillmore et al. 1998). Additionally, non-drinkers have been suggested to differ from drinkers in dietary habits, BMI and physical activity (Barefoot et al. 2002), and in study **III**, the non-drinkers did not suffer an increased cognitive impairment risk when the analyses were controlled for blood pressure, cholesterol level and BMI. Hence, the finding of an increased cognitive impairment risk in non-drinkers must be interpreted with care.

The study protocol made it possible to examine whether genetic factors influence the association between drinking and cognitive impairment. Heavy drinking and multiple pass-outs due to drinking remained significant risk factors for cognitive impairment when also adjusting for the number of ApoE ε4 alleles, whereas non-drinking or binge drinking on at least a monthly basis did not. In the subgroup analyses based on ApoE status, the significant associations between drinking habits and cognitive impairment were limited to subjects without an ε4 allele. Co-twin control analyses including twin pairs clearly discordant in cognitive function permitted an assessment of the association between drinking and cognitive impairment risk with strong control for familial and genetic factors. Even though no statistically significant ORs were seen in these analyses, probably due to low statistical power caused by the small number of suitable twin pairs, the point estimates for heavy drinking and binge drinking were similar to those calculated in the main analyses, suggesting that the elevated risks are independent of family background.

Few studies have assessed the association between midlife alcohol consumption and subsequent dementia risk. The CAIDE study found no association between drinking and dementia risk (Anttila et al. 2004), and the Prospective Population Study of Women also failed to show any association between baseline beer or wine consumption and dementia risk, although continual wine consumption was protective against dementia (Mehlig et al. 2008). However, the assessment of alcohol intake in both of these studies was possibly too crude to allow reliable assessment of any dose effect, as in contrast to study

III, they included only frequency instead of quantity assessment. In a study of Finnish MZ twins, no significant association between midlife total alcohol consumption and dementia risk was seen, but binge drinking was an independent risk factor for dementia. Heavy drinking seemed to increase dementia risk, but the association did not reach statistical significance, possibly because the number of subjects was approximately four times smaller than in study **III**. (Järvenpää et al. 2005)

Previous prospective studies of elderly subjects have mostly found a decreased risk of dementia or AD associated with mild to moderate alcohol consumption (Yoshitake et al. 1995; Huang et al. 2002; Ruitenberg et al. 2002; Mukamal et al. 2003; Simons et al. 2006). Consumption associated with a decreased risk has ranged from ever drinking (Yoshitake et al. 1995) to 7-21 drinks per week (Ruitenberg et al. 2002). However, some studies have failed to detect any protective effect associated with any alcohol consumption (Yip et al. 2006) or drinking 1-7 drinks per week (Truelsen et al. 2002).

There is inconsistency on how the interaction between ApoE genotype and drinking appears to affect the risk of dementia. A four-year long follow-up study of 980 individuals showed a decreased risk of dementia in light to moderate wine drinkers compared to non-drinkers in ε4 non-carriers (HR 0.44), but not in ε4 carriers (Luchsinger et al. 2004). Additionally, in the Cardiovascular Health Study significant associations between drinking and dementia risk were restricted to ε4 non-carriers (Mukamal et al. 2003). However, in the Rotterdam study, a six-year follow-up revealed a reduced risk of dementia associated with light drinking in ε4 carriers but not in non-carriers (Ruitenberg et al. 2002). In addition, the CAIDE study suggested that ε4 carriers drinking several times a month had an increased dementia risk compared to non-drinkers, whereas no such effect was seen in ε4 non-carriers (Anttila et al. 2004).

In study **III** the number of ε4 carriers who reported more than two pass-outs in 1981 was low (n = 8), but the RR point estimate for binge drinking was clearly non-significant. The point estimate associated with heavy drinking was similar to that observed in the main analyses, although it did not reach statistical significance. In contrast, the risk estimate of non-drinkers did not differ from that of light drinkers in ε4 carriers. In subjects without an ε4 allele, all RR estimates were similar to those found in the main analyses. Therefore, study **III** suggests that the association between drinking and cognitive impairment is mainly limited to individuals without an ε4 allele. However, more studies are needed on this interaction, as the differing findings between studies may be attributed to methodological differences (e.g. the age structure and assessment of alcohol consumption as well as the evaluation of cognitive function have differed considerably.)

The mechanisms through which binge drinking independently increases subsequent risk of cognitive impairment may include direct neurodegeneration. In rats, a single four-day long exposure to high blood alcohol levels inflicted damage to the corticolimbic system, including portions of hippocampus (Obernier et al. 2002). This kind of damage would possibly render binge drinkers more vulnerable to AD pathogenesis. Neurodegeneration could result from excitotoxicity and oxidative glutamate toxicity caused by an increased

glutamate release, or deregulation in the inflammatory activation signaling of microglia (Ward et al. 2009). However, it has been shown that 'clinically healthy' abstaining individuals with alcohol dependency or alcoholism perform worse than controls on multiple cognitive domains, including verbal and visual memory, intelligence and frontal lobe functions tests (Davies et al. 2005; Rosenbloom et al. 2005). Additionally, there is evidence that lower cognitive performance in early adulthood increases the risk of alcohol abuse or dependence in midlife (Gale et al. 2008). Hence, it is possible that the association between cognitive function and heavy as well as binge drinking is due to bidirectional effects or even some underlying common liabilities.

In addition, refraining from drinking seems to increase the risk of cognitive impairment when compared to light drinking. This can be attributed to possible protective effects of light to moderate drinking. Non-drinking as well as heavy drinking are risk factors for ischemic stroke (Reynolds et al. 2003), and the underlying mechanisms probably also apply to VCI. Additionally, multiple mechanisms have been postulated for a possible neuroprotective effect of light to moderate alcohol consumption, including anti-inflammatory effects and prevention of excitotoxic damage (Collins et al. 2009).

6.2.2 Midlife cardiovascular risk factors as predictors of cognitive impairment (study IV)

In study **IV**, midlife obesity, hypertension and low physical activity were found to significantly increase the subsequent risk of cognitive impairment over 20 years later when adjusting for age, sex and educational level, but hypercholesterolemia did not seem to be a significant risk factor. The most important determinants seem to be obesity and low leisure time physical activity, since they approximately doubled the risk of cognitive impairment and remained significant risk factors in the model which included all assessed risk factors as well as age, sex and educational level. Midlife overweight (BMI 25-30 kg/m^2) was not associated with a significant risk increase, but using limited follow-up data, further weight gain during midlife did seem to be detrimental in already overweight subjects. Similar follow-up data suggested that development of hypertension early in midlife was especially harmful. In an unadjusted model, all RR point estimates were higher than in the adjusted model, and also hypercholesterolemia was associated with a significantly increased risk, implying that the prevalence of the assessed risk factors differ by age, sex or educational level.

In a model controlling also for ApoE genotype, the risk associated with obesity was clearly attenuated, as was the risk associated with not participating in leisure time physical activity. In contrast, the risks associated with the other assessed factors were not significantly affected. However, as compared to subjects without an ε4 allele, the carriers of an ε4 allele were more often obese (5.7 % and 9.3 %, respectively, p=0.03). No corresponding differences were seen for any other risk factor. The higher prevalence of obesity among the ε4 carriers is probably coincidental as ApoE genotype has not been linked to obesity, but could still explain the attenuation of the risk ratio associated with obesity in the model including ApoE status. In subgroup analyses based on ApoE status, significant risk increases were seen only among the ε4 carriers. This suggests that

cardiovascular risk factors might be especially detrimental for ε4 carriers. However, the finding must be interpreted with care because the interactions between ApoE genotype and the cardiovascular factors were clearly non-significant.

Previous research has suggested that cardiovascular risk factors might be associated with cognitive function especially in ε4 carriers (Zade et al. 2010; Zade et al. 2011). However, these findings are based on cross sectional studies, and none of the studies assessing the association between midlife cardiovascular risk factors and later dementia risk have reported subgroup results based on ApoE status. However, in the CAIDE study no significant interactions were found between ApoE genotype and total cholesterol or blood pressure, and ApoE together with these factors seemed to increase dementia risk in an additive manner (Kivipelto et al. 2002). Therefore, additional studies on the interactions between cardiovascular risk factors and ApoE genotype are needed.

A co-twin control analysis including twin pairs discordant in cognitive function made it possible to rigidly control for genetic and family background. The OR point estimate associated with obesity was clearly attenuated and non-significant in these analyses. In contrast, OR point estimates of other risk factors were not clearly affected, but were statistically non-significant. The lack of statistical significance can probably be attributed to low statistical power caused by the small number of discordant twin pairs. Taken together, the present results suggest that familial factors influence the association between obesity and cognitive impairment risk, whereas other cardiovascular risk factors act more independently of familial factors. The findings of the co-twin control analysis are in line with a previous study based on Swedish twins (Xu et al. 2011), and adjusting for ApoE has also attenuated the risk associated with obesity in previous studies (Kivipelto et al. 2005). The findings in study **IV** are also in line with a study on the same study population concentrating on the cognitive continuum (Laitala et al. 2011).

Multivariate analyses have suggested that midlife hypertension as much as quadruples the dementia risk (Launer et al. 2000), although other studies have hinted at more conservative risk estimates in line with study **IV** (Kivipelto et al. 2001; Whitmer et al. 2005). The association seems to be independent of ApoE status (Kivipelto et al. 2002; Peila et al. 2001). Study **IV** is also in line with previous studies on the association between midlife obesity and dementia or AD (Kivipelto et al. 2005; Rosengren et al. 2005; Whitmer et al. 2007; Xu et al. 2011), and a linear relationship has been proposed between BMI and dementia (Kalmijn et al. 2000). In contrast to study **IV**, some studies have also shown midlife overweight to be a risk factor for dementia and AD (Whitmer et al. 2007; Xu et al. 2011), and a recent meta-analysis supported this finding even though considerable heterogeneity was found in the various studies (Anstey et al. 2011). Previous studies have found leisure time physical activity to be protective of dementia and AD with similar point estimates as in study **IV** (Andel et al. 2008; Chang et al. 2010; Rovio et al. 2005). Similar to study **IV**, studies on discordant twin pairs have failed to reveal any statistically significant associations (Andel et al. 2008; Carlson et al. 2008).

In contrast to obesity, hypertension and low leisure time physical activity, hypercholesterolemia was not a significant risk factor for cognitive impairment in study **IV**. This is inconsistent with previous studies which have detected an elevated risk of dementia or AD associated with total cholesterol levels above 6.2-6.5 mmol/l (Notkola et al. 1998; Kivipelto et al. 2001; Solomon et al. 2009). However, a prospective study on Swedish women failed to observe this association (Mielke et al. 2010). The lack of significant findings in study **IV** may be attributed to methodological shortcomings discussed in detail below. It was not a result of an interaction between cholesterol level and obesity, as the findings were not affected by controlling for BMI (results not shown).

An association was seen between the number of assessed risk factors and cognitive impairment risk in study **IV**, and subjects with at least three of the assessed risk factors had a fourfold risk of cognitive impairment compared to the subjects with no risk factors. As this analysis was controlled for age, sex and educational level, it emphasizes the independent effects of vascular factors. Hence, the accumulation of these easily measurable risk factors seems to increase the risk of cognitive impairment in an additive manner; there are several previous studies supporting this finding. A statistical z score including numerous cardiovascular factors has been shown to correlate with dementia risk (Kalmijn et al. 2000), and an increasing number of different cardiovascular risk factors included in the Framingham Cardiovascular Risk Score increased dementia risk (Whitmer et al. 2005). In the CAIDE study, hypertension and hypercholesterolemia increased the risk of AD in an additive manner (Kivipelto et al. 2001).

6.2.3 Risk score for predicting cognitive impairment risk 23 years later (study IV)

A dementia risk score has been developed based on the CAIDE study; this score has been postulated to predict the dementia risk 20 years later by utilizing easily assessed demographic and cardiovascular characteristics in midlife (Kivipelto et al. 2006). However, the score was developed on the same study population in which it was subsequently tested, and has not been validated in an external population prior to study **IV**. The suggested risk score including age, sex, BMI, blood pressure, cholesterol level and leisure time physical activity was slightly modified in study **IV** to fit the questionnaire data available. Similarly, an alternative score including also the number of ε4 alleles was formed. ROC analyses provide a means to assess the efficacy of prediction so that increasing AUC values indicate better discriminatory ability. In study **IV**, the scores with and without ApoE status had AUC values of 0.74 and 0.75, respectively. These are very similar to those reported in the original CAIDE study, differing by less than five percent from the postulated values. Hence, the risk score seems valid at least in the Finnish population. The predicted probability of cognitive impairment increased with increasing scores, but the number of subjects with maximal or nearly maximal scores was low, resulting in wide confidence intervals in the predicted probabilities of the highest scores. In order to further emphasize the significance of the findings, risk score quartiles were compared in study **IV**, and subjects in the highest risk

score quartiles had 9-11 times higher risks of suffering cognitive impairment as compared to subjects in the lowest quartiles.

The best known risk scores are probably the Framingham Heart Study risk model (Anderson et al. 1991) and the SCORE system (Conroy et al. 2003), which are used in the clinics to predict an individual's cardiovascular disease risk. Hence, they provide a basis for reflecting the clinical significance of the dementia risk score. AUC values for the Framingham model and SCORE are approximately 0.10 higher than those found for the dementia risk score, indicating higher accuracy (Scheltens et al. 2008). Therefore, the dementia risk score needs further evaluation and possibly refinements before it can be introduced into clinical practice. However, because dementia has a multifactorial origin and the score aims to predict dementia two decades later, it might not be possible to gain higher accuracy. The lack of significant improvement in accuracy by the inclusion of ApoE into the score may reflect this.

Even in its current form, the risk score could represent one additional tool for physicians trying to encourage patients to comply with preventive measures and treatment of cardiovascular risk factors. The contribution of cardiovascular risk factors on dementia incidence has been modelled using RR and prevalence estimates retrieved from the published literature, and a ten percent reduction in the prevalence of midlife hypertension could potentially prevent 160 000 AD cases around the world during the next 40 years. Similar reductions in the prevalences of midlife obesity and physical inactivity could prevent 167 000 and 380 000 cases, respectively. (Barnes and Yaffe 2011)

In contrast to the accumulating evidence on the association between cardiovascular risk factors and dementia, the number of studies estimating the influence of treating these factors on the risk of dementia is limited. There is some evidence from prospective cohort studies that the risk associated with hypertension is attenuated by antihypertensives (Launer et al. 2000). A systematic review of controlled trials conducted in 2003 concluded that treatment of hypertension was not associated with a significantly reduced dementia risk, but in studies using a dihydropyrine or diuretic as the mainstay of therapy a significant risk reduction was achieved (Wang et al. 2003). More recently, a Cochrane review analysed four randomized controlled trials on elderly hypertensive subjects without cerebrovascular disease. The mean planned length of study was five years, and the authors found no convincing evidence that blood pressure lowering in late-life could prevent the development of dementia. (McGuinness et al. 2009) A prospective study with a mean follow-up of nine years found that statin use was associated with a decreased dementia risk, but the association was statistically significant only when comparing any use with no use of statins (Haag et al. 2009). Two randomized controlled trials have concluded that statin treatment in late life has no effect in preventing dementia (McGuinness and Passmore 2010). However, all of the mentioned randomized controlled trials have examined subjects who were elderly already at baseline, and therefore their results may not be applicable to middle aged subjects.

6.2.4 Changes in cerebrovasculature as a common pathway?

Multiple mechanisms can underlie the increase in the risk of dementia caused by cardiovascular risk factors. These factors have been postulated to increase the risk of cerebrovascular disease and VCI (Gorelick 1997). However, as 'pure' VCI only accounts for a minority of dementia cases, it is unlikely to completely explain the association. Instead, for the risk factors to have such a significant effect they must also affect the risk of clinical AD.

A significant proportion of AD patients display findings of cerebrovascular pathology post-mortem (Jellinger 2006), and the most common neuropathological finding in community-dwelling older subjects with dementia has been suggested to be AD pathology with infarcts (Schneider et al. 2007). Cerebrovascular disease (including infarcts, lacunes and subcortical small-vessel disease) also lowers the threshold at which AD pathology causes clinical dementia (Snowdon et al. 1997; Esiri et al. 1999). A total of 156 subjects with mixed dementia who had Clinical Dementia Rating Scale performed at most three months prior to death and who were free of other neurological disorders, macroscopic infarcts and non-AD related pathology were subjected to neuropathologic evaluation post mortem. A vascular score was found to explain 15 % of the variability in the presence of dementia, whereas Braak NFT staging and Aß explained 30.4 % and 3.5 %, respectively. (Gold et al. 2007) Interestingly, a U-shaped association between alcohol consumption and white matter changes in MRI has been postulated, so that drinking 1-7 drinks per week is associated with the fewest changes (Mukamal et al. 2001).

It seems plausible that both cardiovascular risk factors and alcohol consumption exert their effects on the risk of cognitive impairment through changes in the cerebrovasculature, possibly thus rendering subjects more vulnerable to further brain pathology. ApoE as well as other suggested genetic risk factors or candidate genes for AD are associated with cardiovascular disease, so the association between cerebrovasculatur pathology and AD may be evident even at the level of molecular genetics (Rocchi et al. 2009). However, the evidence described above can be interpreted in two ways. Some authors argue that vascular changes are actually causative of AD pathology (de la Torre 2002). However, it is equally plausible that AD pathology, including Aβ and NFTs, and vascular pathology represent independent pathogenetic mechanisms in a large proportion of patients, and that their interaction or additive effects ultimately impair cognition to the degree of clinical dementia.

Different cardiovascular risk factors interact significantly with each other and probably with socioeconomic factors as well, but it is possible that obesity and leisure time physical activity both have central nervous system effects independent of these interactions. Metabolic changes associated with obesity might impair synaptic plasticity in hippocampus (Harvey 2003). Obesity and excess adipose tissue are often associated with hyperinsulinemia and insulin resistance, and there is evidence suggesting that this can lead to an accumulation of AD pathology partly due to inflammatory mechanisms (Craft 2005). Physical activity is considered to be associated with increased expression

of brain growth factors, and may also be neurogenerative and neuroprotective against ischemic or neurotoxic damage (Dishman et al. 2006). Study **IV** supports the conception that the protective mechanisms of physical activity might not be entirely vascular, as belonging to the first MET index quartile remained a significant risk factor in a model including all the assessed cardiovascular risk factors and borderline significant in a model controlling for the number of other risk factors.

6.2.5 Methodological considerations in studies III and IV

Studies **III** and **IV** consisted of follow-up of about 2 000 subjects for 23 years. The subjects were middle-aged at baseline, and therefore early risk factors for cognitive impairment could be detected. As stated above, this is imperative since the pathogenesis of AD is considered to begin over a decade before clinical manifestation. The study population consisted of twins from the older Finnish Twin Cohort, and can be assumed to represent the general Finnish population. All the statistical analyses took into account the possible clustering caused by the twin design. Additionally, interactions between genetic factors and the assessed risk factors could be studied as ApoE status was available for the majority of subjects, and the twin population permitted separate co-twin control analyses on pairs discordant for cognitive impairment, providing further control for genetic and family background. The reported statistical models did not control for follow-up, but the results were similar when follow-up was included in the models. Only in the co-twin control analysis, the risk associated with obesity was affected by controlling for follow-up, becoming more similar to the main analyses.

Previous studies examining the association between drinking and dementia have used varied categorization criteria of alcohol consumption. In the current study, alcohol consumption categories were based on calculated daily consumption converted into drinks per week, with the categories following proposals by the National Institute of Alcohol Abuse and Alcoholism (National Institute of Alcohol Abuse and Alcoholism 2010). In addition, the drinking pattern was estimated; few previous studies have assessed its effect on the risk of cognitive impairment or dementia. Unfortunately, the study design did not allow assessment of beverage specific effects, even though there is some evidence that especially wine consumption is protective of dementia (Truelsen et al. 2002; Mehlig et al. 2008). However, wine accounted for only about 10-15 % of total absolute alcohol consumption in Finland at the time of the baseline assessment (National Institute for Health and Welfare 2011).

The assessment of alcohol consumption was based on self-report, and therefore was not free of possible bias. There is a general consensus that self-reported assessment of drinking has adequate reliability and validity for most research purposes, even though there is considerable within-person variability in drinking patterns and questions without a defined time-frame may be inferior to those with a definite time-frame (Del Boca and Darkes 2003). A study on twins living in Finland and Sweden used a questionnaire identical to that applied in study **III** to assess daily consumption, and compared the results to biochemical markers of alcohol consumption and dietary history interviews. Subjects reporting a higher intake in the questionnaire had higher levels of the

biochemical markers than subjects reporting lower intake, but the relationship weakened with increasing reported intake. In addition, the questionnaire identified far fewer heavy drinkers than the dietary history interviews. The authors concluded that only strong associations between drinking and some particular disorder are likely to be detected if the data are based on questionnaire material. (Carlsson et al. 2003) This would – if anything – render the risk estimates in study **III** as being too conservative. However, it is possible that subjects classified as heavy drinkers actually drink considerably more than they report, which makes it difficult to find an intake level beyond which drinking becomes harmful.

Similarly to the situation with alcohol consumption, also the assessment of cardiovascular risk factors in study **IV** was based on self-report instead of direct measurement. However, the correlation between self-reported and a measured BMI 4-5 years later has been shown to be approximately 0.90 (Korkeila et al. 1998), and thus self-report is unlikely to have affected the results with regard to BMI. In the assessments of hypertension and hypercholesterolemia, the subjects were asked to report the presence of hypertension during the five years preceding the questionnaire, and therefore were liable to some bias. A significant number of subjects had unknown blood pressure or cholesterol levels, but the exclusion of such subjects from the analyses did not affect the results. Subjects with questionnaire-reported use of antihypertensives have been shown to have significantly higher measured blood pressure than subjects reporting no use (Hernelahti et al. 1998), and in a study of 596 Finnish middle-aged and elderly subjects, the prevalence of self-reported hypertension correlated strongly with medical record data (Haapanen et al. 1997). Unfortunately, questionnaire data on hypercholesterolemia have not been formally validated against measured values or medical records. The effects of exposure time could be assessed, since follow-up data from 1981 to 1990 were available for a part of the subjects. Treatment effects could not be studied, but in the 1980s hypertension and hypercholesterolemia were and could not be treated as aggressively as today. In addition, studies **III** and **IV** included only subjects alive at the end of follow-up, so a possible role of survival bias cannot be excluded, as e.g. Mielke et al. (2010) have suggested.

In the analyses controlling for ApoE status or the number of cardiovascular risk factors in addition to age, sex and educational level, there was some discrepancy between the two variables assessing physical activity. The results for being categorized as being physically sedentary were attenuated in these analyses, whereas the results for the lowest MET index quartile were not. The number of subjects classified as occasional exercisers was high (82.1 %), and the low number of subjects in the two other groups add uncertainty to the results. This is reflected by the long confidence intervals in these analyses. Therefore, the MET index can be seen as a more reliable and robust estimator of physical activity.

In the evaluation of the suggested dementia risk score in study **IV,** the original score had to be modified to fit the questionnaire data available. Identical categorizations could be used for age, sex, educational level, BMI and ApoE status, and the differences in

physical activity categorization were minor between the proposed and modified scores. However, it must be noted that the categorization criteria for hypertension and hypercholesterolemia did differ, and are prone to the problems mentioned above. However, due to stricter diagnostic criteria for both hypertension and hypercholesterolemia in the 1980s, the categorizations in the modified risk scores are probably adequate. A large number of subjects with either MICF or missing information on one or more of the risk factors had to be excluded from the risk score analyses, but the subjects who remained for analysis did not differ greatly from the excluded subjects, suggesting that this group was not selected.

The assessment of cognitive function with a telephone interview has some practical limitations, including possibly impaired hearing ability, and difficulties in assessing certain cognitive functions without actually seeing the subject. It is also impossible to verify that the subjects do not use calendars or newspapers as sources of orientation. Careful measures were implemented to attempt to overcome these and other shortcomings, for example by asking whether the subject could hear well and whether he/she was feeling rested, and by forbidding the use of pens, pencils, papers, newspapers and calendars. (Järvenpää et al. 2002) The TELE has been validated and shown to differentiate subjects with mild to moderate AD from healthy controls, and the TELE score correlates with clinical dementia severity. The chosen cut-off points have been shown to possess high specificity in the cognitively intact and impaired groups. (Gatz et al. 1995; Järvenpää et al. 2002)

The study did not include a clinical diagnostic work-up for dementia. Hence, the presented findings cannot be directly interpreted to apply to dementia, but there are no obvious reasons why the results would be systematically or significantly different if clinical diagnoses had been available. The lack of clinical diagnostic work-up did, however, prevent separate analyses for AD and VCI. Cognitive function was not assessed at baseline, so it could be argued that some degree of early cognitive impairment in some participants could have affected the results. This is however extremely unlikely, as AD usually develops after the age of 65 years, and less than three percent of the interviewed subjects were older than this in 1981. In addition, the results regarding either midlife alcohol consumption or cardiovascular risk factors did not change when all of those subjects older than 55 in 1981 were excluded from the analyses (results not shown).

7. CONCLUSIONS

The main conclusions of the presented studies can be summarized as follows:

I   Non-demented co-twins of monozygotic twin pairs discordant for Alzheimer's disease have a lower regional cerebral glucose metabolic rate compared to unrelated controls. The reductions involve but are not restricted to regions usually affected by Alzheimer's disease. In contrast, no such reductions are seen in non-demented co-twins of similar dizygotic twin pairs. This suggests that the reduced glucose metabolic rate is caused by genetic factors, and is a possible indicator of an elevated genetically mediated risk of Alzheimer's disease, or of genetically mediated background hypometabolism rendering individuals vulnerable to the pathogenesis of Alzheimer's disease.

II   Heavy alcohol consumption and binge drinking in midlife increase the risk of cognitive impairment over 20 years later. The risk increase associated with binge drinking is independent of total alcohol consumption. Light drinkers may have a reduced risk of cognitive impairment as compared to non-drinkers, but this finding must be interpreted with care. The elevated risks associated with heavy and binge drinking seem to be independent of familial factors, but the associations between drinking and cognitive impairment risk may differ depending on the apolipoprotein E genotype.

III   Midlife obesity, hypertension and low leisure time physical activity increase cognitive impairment risk over 20 years later, but hypercholesterolemia does not elevate the risk. Obesity and low physical activity are the most significant risk factors. The effects of these risk factors seem greater in subjects with at least one ε4 allele, but this finding must be interpreted with care.

IV   The accumulation of midlife cardiovascular risk factors increases cognitive impairment risk in an additive manner. A previously devised risk score for predicting dementia 20 years later is able to predict cognitive impairment well also in an external study population, but its accuracy is not good enough to allow it to be used in clinical practice without further testing and potential refinements. However, the additive effects of cardiovascular risk factors emphasize the importance of their prevention and appropriate treatment.

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