DEPARTMENT OF NEUROLOGY SERIES OF REPORTS NO 88, 2007

SANNA-KAISA HERUKKA

Usefulness of Cerebrospinal Fluid Biomarkers in Diagnosis of Early Alzheimer's Disease

Doctoral dissertation

To be presented with assent of the Medical Faculty of the University of Kuopio for public examination in Auditorium ML1, Medistudia building, University of Kuopio, on Friday 31st August 2007, at 12 noon

Department of Neurology
University of Kuopio

Department of Neurology
Kuopio University Hospital

Distributor: Department of Neurology
University of Kuopio
P.O. Box 1627
FI-70211 KUOPIO
FINLAND
Tel. +358 17 162 682
Fax +358 17 162 048

Author's address: Department of Neurology
University of Kuopio
P.O. Box 1627
FI-70211 KUOPIO
FINLAND
Tel. +358 17 163 213
Fax +358 17 162 048

Supervisors: Professor Tuula Pirttilä, M.D., Ph.D.
Department of Neurology
Kuopio University Hospital

Professor Heikki Tanila, M.D., Ph.D.
Department of Neurobiology
University of Kuopio

Reviewers: Professor Irina Elovaara, M.D., Ph.D
Department of Neurology
University of Tampere

Professor Juha Rinne, M.D., Ph.D
Turku PET Centre
University of Turku

Opponent: Professor Gunhild Waldemar, M.D., D.M.Sc
Department of Neurology
Copenhagen University Hospital
Denmark

ISBN 978-951-27-0820-8
ISBN 978-951-27-0218-3 (PDF)
ISSN 0357-6043

Kopijyvä
Kuopio 2007
Finland

Herukka, Sanna-Kaisa. Usefulness of cerebrospinal fluid biomarkers in diagnosis of early Alzheimer's disease. Series of reports, No. 88, Department of Neurology, University of Kuopio 2007. 87 p.
ISBN 978-951-27-0820-8
ISBN 978-951-27-0218-3 (PDF)
ISSN 0357-6043

ABSTRACT

Alzheimer's disease (AD) is the most common dementing disease and the number of patients with AD is increasing as the population is ages. AD not only has a devastating effect on individual patients and their families, but it also poses an enormous socioeconomic burden to society. AD is a disease that develops slowly over a time range of two decades before the appearance of first symptoms. The neuropathology starts to develop during the preclinical period of the disease, so that at first symptomatic stage the pathology is already advanced. This is a major obstacle in treating the disease because at present there is no way known to repair nerve damage once it has occurred. Previous studies indicated that levels of CSF biomarkers Aβ42, tau and phospho-tau are altered in AD patients in comparison to healthy controls or patients with other neurological diseases and they can be used as a diagnostic aid, especially in clinically challenging conditions, for example in the differentiation between mild AD and depression.

At the beginning of this study, little was known about these biomarkers before the onset of clinical AD. Since AD neuropathology starts to develop decades before symptoms, the original hypothesis was that AD associated changes in CSF biomarkers could be detected before the appearance of clinical AD. The main aim of the study was to examine whether these changes would predict the development of AD in patients with mild cognitive impairment (MCI) who have a high risk for of suffering AD within a period of a few years. The association between AD associated pathological changes in brain and the CSF biomarker levels were also examined.

The results of this study revealed that the AD associated changes in CSF biomarkers, i.e. decrease in Aβ42 and increase in tau and phospho-tau occurred during the predementia stage of AD before the clinical AD diagnosis. CSF Aβ42, tau and phospho-tau values were also associated with medial temporal lobe atrophy. The combination of decreased CSF Aβ42 and increased tau or phospho-tau could differentiate MCI patients who would develop AD from those who remained stable with high specificity even several years before the clinical diagnosis of AD. These results suggest that the abnormal CSF biomarker levels can be used for confirmation of a clinical diagnosis of possible early AD. However, the sensitivity was low and therefore a negative result cannot exclude presence of AD. A large proportion of MCI patients did not exhibit memory impairment. Executive dysfunction without amnestic symptoms was common, and these patients developed AD almost as frequently as MCI patients with memory impairment.

In conclusion, the pathological changes of the biomarker levels occur early during the course of the disease, and can predict development of AD in patients with cognitive impairment. In the clinical context, patients with cognitive impairment without memory complaint should also be considered to be at high risk for developing AD.

National Library of Medicine Classification: WT 155, WL 359, WL 141, WL 203, QY 220
Medical Subject Headings: Alzheimer Disease/diagnosis; Brain/pathology; Cognition Disorders; Disease Progression; Memory; Cerebrospinal Fluid; Biological Markers; tau Proteins; Amyloid beta-Protein; Temporal Lobe/pathology

To dare is to lose one's footing momentarily.
To not dare is to lose oneself.

-Soren Kierkegaard-

ACKNOWLEDGEMENTS

This work was carried out in the Department of Neurology, University of Kuopio and Department of Neurology, Kuopio University Hospital during the years 2001-2007.

I wish to express my warmest gratitude to my principal supervisor Professor Tuula Pirttilä for her continuous guidance and support during these years. She has such a passion for her work which I hope has rubbed off on me, to be there during what I hope will be my future in science. Tuula has given me space to learn by working independently and trying to solve problems for myself - sometimes even by making mistakes. At the same time, I always sensed her encouragement and she was always there when needed. You made me feel that you had confidence in me, even in off-days, and it helped me to feel more confident about myself too. You have been an excellent mentor, and I hope that one day I will be able to pass on things I have learned from you to benefit someone else.

I wish to thank my supervisor Professor Heikki Tanila for introducing me to the field of neuroscience and showing great patience when I was still a novice. You have a way of lifting a student's spirits when things don't go as planned. Bad results or a failed experiment never looked so awful after talking with you.

I owe my deepest gratitude to Professor Hilkka Soininen, the head of Department of Neurology, for the opportunity to carry out this work under such excellent facilities. I would also like to thank you for your support, encouragement and helpful advice during these years.

I am very grateful to the official reviewer of this thesis, Professor Irina Elovaara and Professor Juha Rinne, whose careful examination and constructive comments helped me to substantially improve the manuscript.

I am thankful to my co-authors Li Liu, Rimante Minkeviciene, Thomas van Groen, Merja Hallikainen, Seppo Helisalmi, Susanna Tervo and Corina Pennanen for their collaboration.

I wish to thank Ewen MacDonald for revising the language of this thesis.

My most sincere thanks belong to Päivi Räsänen, whose endless encouragement has helped me throughout these years. You are the kind of a person that halves one's sorrows but doubles their happiness after sharing them with you. I extend my warmest thanks to the rest of our one-o-clock coffee team, Marja-Leena Soininen and Tiina Suuronen for giving me much-needed extra energy for the afternoons. I also thank Seija Hynynen and Marjo Laitinen for their excellent technical assistance. I am also grateful to Esa Koivisto, Sari Palviainen, Tuija Parsons, Nilla Nykänen, Mari Tikkanen and Tuula Toivanen for their invaluable help. In addition to these people, I would like to thank all the personnel of the Department of Neurology.

I would also like to thank all the former and present students of the Department of Neurology. Particularly I would like to express my gratitude to Tero Tapiola and Petri Kerokoski for tutoring me in many technical problems, especially when I had just begun my career in neuroscience. My warmest thanks belong also to the members "Misukat", Anne Hämäläinen, Hennariikka Iivonen, Minna Korolainen, Mari Oksman, Laura Parkkinen and Mia Pirskanen,

for your friendship and many memorable moments during the years that I have known you.

I would like to express my deepest gratitude to my family, my mother Tyyne Herukka and brothers Lauri and Ilkka Herukka. You have effectively kept my mind off the work and my feet grounded whenever visiting you. You have also taught me the value of good work ethics, always giving my best.

There are many people, dear friends, colleagues and relatives, I haven't been able to mention here but who are definitely worth a great big thanks. I have not forgotten you; you are always in my heart.

Finally, I would like to thank all the patients and their caregivers who participated in the study. Without these people's altruism, this work would not have been possible.

The study was supported by Department of Neurology, University of Kuopio, Drug Discovery Graduate School, Academy of Finland, Kuopio University Hospital (EVO grants 5883, 577270, 5772722, 5772725), Nordic Center of Excellence in Neurodegeneration, The Finnish Alzheimer Research Society, The Research and Science Foundation of Farmos, The Lilly Foundation, The Emil Aaltonen Foundation, The Päivikki and Sakari Sohlberg Foundation, The Maud Kuistila Foundation, The Neurology Foundation, The Finnish Medical Foundation and The Finnish Cultural Foundation of Northern Savo.

Kuopio, August 2007

Sanna-Kaisa Herukka

ABBREVIATIONS

AACD	age-associated cognitive decline
AAMI	age-associated memory impairment
Aβ	amyloid-beta peptide
AD	Alzheimer's disease
ApoE	apolipoprotein E
APP	amyloid precursor protein
AUC	area under curve
BBB	blood brain barrier
CDR	clinical dementia rating
CIND	cognitive impairment no dementia
CJD	Creutzfedt-Jacob disease
DLB	dementia with Lewy bodies
DS	Down syndrome
EC	entorhinal cortex
FD	frontal degeneration
FTD	frontotemporal dementia
HC	hippocampus
LR	likelihood ratio
MCI	mild cognitive impairment
MMSE	mini mental state examination
MTL	medial temporal lobe
NAS	non aliter specificatus
NFT	neurofibrillary tangle
NINCDS-ADRDA	National Institute of Neurologic and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association
NSAID	non-steroidal anti-inflammatory drug
OR	odds ratio
PET	positron emission tomography
PIB	Pittsburgh Compound-B
PS	presenilin
ROC	receiver operator characteristics
SPECT	single photon emission computed tomography
TE	time of echo
TR	time of repetition
VaD	vascular dementia
VCI	vascular cognitive impairment

LIST OF ORIGINAL PUBLICATIONS

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

I Liu L, Herukka S-K, Minkeviciene R, van Groen T, Tanila H (2004). Longitudinal observation on CSF Aβ42 levels in young to middle-aged amyloid precursor protein/presenilin-1 doubly transgenic mice. *Neurobiology of Disease* 17. 516-523

II Herukka S-K, Hallikainen M, Soininen H, Pirttilä T (2005). CSF Aβ42 and Tau or phosphorylated Tau and prediction of progressive mild cognitive impairment. *Neurology* 64, 1294-1297

IIIa Herukka S-K, Helisalmi S, Hallikainen M, Tervo S, Soininen H, Pirttilä T (2007). CSF Aβ42, Tau and phosphorylated Tau, APOE ε4 allele and MCI type in progressive MCI. *Neurobiology of Aging 28, 507-514*

IIIb Herukka S-K, Helisalmi S, Hallikainen M, Tervo S, Soininen H, Pirttilä T (2007). Response to: "Heterogeneity of mild cognitive impairment and other predementia syndromes in progression to dementia". *Neurobiology of Aging* (in press)

IV Herukka S-K, Pennanen C, Soininen H, Pirttilä T. CSF Aβ42, Tau and phosphorylated Tau correlate with medial temporal lobe atrophy. Submitted for publication

1. INTRODUCTION

The proportion of the elderly is growing steadily as the longevity of the population increases. This leads to greater numbers of patients suffering age-associated neurodegenerative diseases, including dementia. Dementia not only has a devastating effect on the individual patients and their families but it also imposes enormous socioeconomic burden on society. It is estimated that in 2005 there were over 29 million patients with dementia and the worldwide societal costs of dementia were US$315 billion (Wimo et al. 2007). Over half of the dementia cases are caused by Alzheimer's disease (AD). As the treatments for AD evolve, it will become increasingly important to diagnose AD patients as early and as accurately as possible.

Diagnosis of possible or probable AD is based on the clinical criteria and exclusion of other possible causes for dementia. The criteria include an insidious onset and progressive impairment in memory and other cognitive functions severe enough to cause a dementia syndrome. This is a significant problem, since AD has a preclinical period which may last over two decades. At the time of diagnosis using current criteria, the AD associated neuropathology is already well advanced. Therefore the focus of research on AD diagnostics has shifted to the time when the patient exhibits the first clinical symptoms of the disease, usually memory impairment, but the symptoms are not severe enough to validate a diagnosis of AD. These studies led to development of the concept of mild cognitive impairment (MCI), which refers to a transitional stage between cognitively healthy individuals to patients with clinically diagnosable AD (Flicker et al. 1991). Numerous different diagnostic criteria for MCI have been suggested, but it has become evident that MCI patients represent a very heterogeneous group with different etiologies and prognoses.

A definite diagnosis of AD requires a *post mortem* neuropathological examination of brain. The neuropathological hallmarks of AD include the neurofibrillary tangles (NFT) consisting of hyperphosphorylated tau protein and amyloid plaques consisting of amyloid-beta (Aβ) peptide. At the advanced stage of AD, the clinical diagnosis is relatively accurate, but the early diagnosis of AD is challenging. AD associated pathology is also common in patients with other dementing diseases. It is difficult to identify patients with mixed pathology using the current clinical methods. The hypothesis was that since both Aβ and tau can be detected in CSF these proteins hold potential significance as biomarkers for AD. After numerous studies

determining the levels of Aβ42 (the more amyloidogenic form of Aβ), tau and hyperphosphorylated tau (phospho-tau) in AD patients and comparing these values with healthy controls and patients with other neurological diseases, it became evident that CSF levels of Aβ42 are decreased whereas those of tau and phospho-tau are increased in AD (Blennow and Hampel 2003).

Even though the CSF Aβ42, tau and phospho-tau were established as the most promising biomarkers for AD, there were still many unanswered questions. Most studies have been cross-sectional studies performed in clinical patient series. Therefore the actual association between the neuropathologically confirmed diagnosis and CSF biomarkers was uncertain. Also the mechanisms responsible for the changes in CSF and the association between other AD associated pathological changes for example medial temporal lobe (MTL) atrophy were unclear. Furthermore, the AD patients in these studies had relatively advanced disease and there was no data available on CSF Aβ42, tau and phospho-tau from the preclinical period of AD. A few small studies including MCI patients had been published, but the heterogeneity of MCI complicates the interpretation of the results of cross-sectional studies. In addition, the follow-up period needs to be several years to reliably detect those MCI patients who develop AD. In this context the present series of studies was initiated, to study the predictive value of CSF biomarkers for the development of dementia in patients with MCI and to examine the association between the CSF biomarkers and AD associated pathological changes.

2. REVIEW OF THE LITERATURE

2.1 ALZHEIMER'S DISEASE IS A STAGE CONCURRENT DISORDER

When Alois Alzheimer described his patient, Auguste D, about 100 years ago, the syndrome that was later named AD was thought to be a rare disease causing presenile dementia. Subsequently, it was revealed that AD is in fact the most common form of dementia, responsible for over half of all dementia cases. The rare early onset AD is caused by dominant mutations and this form runs in families, whereas in most of the cases, the onset of the disease occurs later, after the age of 65 years. There are genetic risk factors also for late onset AD but the late onset AD genes are not causative factors, they only increase the risk for AD.

Dementia is a syndrome that is defined by a decline in multiple cognitive domains so severe to cause impairment in functional abilities and difficulties in activities in daily living. These symptoms may be caused by different etiologies, but neuropsychiatric causes such as depression and delirium must be excluded. The diagnosis of dementia is currently based on DSM-IV (American Psychiatric Association 1994). AD is a slowly progressing disease that is currently diagnosed based on the clinical criteria proposed by the National Institute of Neurologic and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al. 1984). The criteria include insidious onset, progressive impairment in memory and other cognitive functions plus exclusion of other possible causes for dementia. Clinical work up includes taking medical history and performing medical, neurological and neuropsychological examination. Routine blood tests are done to exclude some treatable causes of dementia, for example B12 vitamin deficiency. Brain imaging, usually CT scan, is done to exclude for example vascular disease and normal-pressure hydrocephalus. The objective of the clinical assessment is to exclude the possibility of clinical conditions other than AD.

The final clinical diagnosis can be either possible or probable AD, since the definite AD diagnosis is made by histopathological analysis of brain tissue obtained either by biopsy or during autopsy. The neuropathological changes in AD brain include amyloid plaques and NFTs, accompanied by neuronal death and synaptic loss in the affected areas. The initial

neuropathological changes may occur as early as two decades before the onset of clinical symptoms (Selkoe 2001).

2.1.1 Neuropathological changes

2.1.1.1 Amyloid pathology

The quintessential feature of AD pathology is the presence of amyloid plaques in the cortical areas of the brain. The main component of the amyloid plaques is Aβ peptide (Masters et al. 1985). The Aβ peptide is a derivative of a larger cell membrane spanning protein, amyloid precursor protein (APP). The Aβ peptide is formed by sequential cleavages of the APP, and it occurs as peptides with different numbers of amino acid residues. The most extensively studied species of Aβ peptides are the peptides containing 40 (Aβ40) and 42 (Aβ42) amino acid residues. Both forms occur in amyloid plaques, but the Aβ42 is deemed to be more amyloidogenic and therefore more damaging to the brain.

The formation of amyloid plaques is thought to be initiated by Aβ42 but subsequently also Aβ40 starts to accumulate (Iwatsubo et al. 1994). The diffuse plaques occur early in the course of the disease. They can be detected only by antibodies staining Aβ and they are not surrounded by other neuropathological changes. Dense amyloid plaques contain fibrillar Aβ and also several other proteins, for example apolipoprotein E (ApoE). They can be detected by stains that are used for all amyloids found in the body, for example thioflavin-S. The dense plaques are also associated with dystrophic neurites and inflammatory processes.

The search for the AD causing gene was facilitated by the finding that patients with Down syndrome (DS), who have a third copy of chromosome 21, develop AD type amyloid pathology at an early age. This association located the APP gene into chromosome 21 and in time, it was characterised and mutations in the APP gene were confirmed to cause early onset AD (Goate et al. 1991). There are several isoforms of APP, but the main isoform found in neurones is APP_{695} and this includes the Aβ sequence. It has been suggested that there are physiological and pathological metabolic routes for APP. When APP is cleaved by α-secretase, which cleaves APP from the middle of the Aβ sequence, production of Aβ peptide is prevented (Haass 2004). During the formation of Aβ peptide, the APP is cleaved by β- and

γ-secretases (Haass 2004, Vassar et al. 1999, Wolfe 2006). All of the currently known mutations that cause familial AD are found in the APP gene or the genes encoding presenilin 1 or 2. The presenilin 1 and 2 are essential parts of the γ-secretase complex and are thus crucial in determining the length of the generated Aβ peptide (Wolfe 2006). All familial AD mutations, except the Arctic APP mutation, selectively increase the formation of Aβ42 and therefore may promote fibrillation of the Aβ into amyloid plaques (Scheuner et al. 1996). The Arctic mutation lowers secreted levels of both Aβ40 and Aβ42 (Nilsberth et al. 2001). The intracellular location of APP is altered so that the generated Aβ remains in the intracellular compartment and there is less available APP for production of secreted Aβ (Sahlin et al. 2007).

One proposed mechanism for development of AD neuropathology is the so-called amyloid cascade hypothesis (Hardy and Selkoe 2002). Not only the above mentioned findings but also the vast number of subsequent studies on APP metabolism in AD explains why this theory is the most popular (but not the only) mechanism to explain the primary pathogenesis of AD. The pathological cascade begins when some factor, for example a genetic risk factor, aging or an environmental factor, increases the formation of Aβ42 or decreases its clearance from the brain. Should the initiating factor be a causative dominant genetic mutation, then the Aβ42 load increases rapidly and the onset of symptoms happens at an early age. In late onset, sporadic AD, the progression of the pathology is slower and the symptomatic stage is reached later. Surprisingly, it was revealed that neither Aβ itself nor the accumulated Aβ plaques were particularly toxic to neurones. Instead, Aβ becomes toxic only after monomeric Aβ accumulates into oligomers (Walsh et al. 2002). These oligomers then induce an immunological response and mediate oxidative stress and impair the function of the affected neurones. Once a threshold in the pathology is passed, neuronal dysfunction and loss cause the first cognitive symptoms. Recently, a specific oligomer that includes twelve Aβ42 units was found to be the main culprit in causing the toxic effects of Aβ in transgenic mice (Lesne et al. 2006). If this finding is corroborated by studies on humans, this might offer a new target molecule for both diagnosis and treatment of AD. The significance of intracellular Aβ in the pathological process is somewhat unclear, but it is possible that it also contributes to the pathology.

2.1.1.2 Tau pathology

In neurones, the tremendous length of the axon when compared to cell body complicates the maintenance of normal function of synapse. Therefore, neurones have very sophisticated machinery to guarantee a functional gateway between the body and periphery of the cell. This consists of microtubules that run along the axon and several different proteins that are involved in the trafficking of essential particles from the cell body to the synapse and back from synapse to body. These particles include cell organelles such as mitochondria and vesicles containing proteins derived from golgi, e.g. tau is one of these microtubule-associated proteins. The main function of tau is to stabilize the microtubules in axons but it also reduces binding of a motor protein called kinesin to microtubules, which in turn obstructs axonal transport (Ebneth et al. 1998). Impaired axonal transport by tau hinders the growth of the neurites and makes them more vulnerable to oxidative stress (Stamer et al. 2002). These conflicting effects of tau on the wellbeing of neurone mean that tau is a metabolically dynamic protein. Under normal conditions, tau is constantly being phosphorylated and dephosphorylated and there is a physiological equilibrium between these two processes.

Tau is expressed in several isoforms of different lengths (Goedert et al. 1989). Phosphorylation of tau is possible at 79 serine and threonine residues, at least thirty of which have been shown to be associated with abnormal phosphorylation in AD (Gong et al. 2005). Phosphorylation of tau inhibits its binding to microtubules and hyperphosphorylation of tau makes it neurotoxic (Fath et al. 2002). Hyperphosphorylated tau is also more resistant to proteases and this makes it more prone to aggregate (Alonso et al. 2001, Johnson et al. 1989). Eventually, hyperphosphorylated tau forms intraneuronal bundles of fibrils called paired helical filaments (Grundke-Iqbal et al. 1986) and after the affected neurone dies, only NFTs are left behind and the second characteristic hallmark of AD neuropathology has been generated.

NFT pathology in the brain occurs in a well documented sequential manner (Braak and Braak 1991). The first changes can be seen in entorhinal cortex (EC) and at that stage the patients do not have any cognitive impairment. When the disease progresses to a stage at which the patient starts to exhibit cognitive impairment, the pathology has spread to hippocampus (HC). Later on, the density of NFTs in these areas increases and the pathology spreads to amygdala and limbic nuclei of the thalamus. Finally, the pathology can be detected in neocortical areas and at this point the patients usually fulfil the clinical criteria for AD. The progression in the

pathology is accompanied by loss of cholinergic neurones in a similar sequential manner. The loss of cholinergic innervation starts from MTL, progresses to adjacent limbic areas and finally to cerebral cortex (Mesulam 2004).

There has been an intense debate about which of these pathological processes, amyloid or tau associated neuropathology, is the initiator of AD pathology. The amyloid cascade hypothesis states that Aβ42 oligomerization and accumulation occur first, and the tau pathology and neuronal dysfunction are downstream events. Previous studies have shown that Aβ accumulation induces phosphorylation of tau (Alvarez et al. 1999) and mice carrying both mutant tau and APP develop more abundant NFT pathology in those brain areas that express both APP and tau in comparison to those areas which express only tau (Lewis et al. 2001). Furhermore, the injection of Aβ fibrils into the brains of mutant tau transgenic mice induces the formation of NFTs (Götz et al. 2001). Support for the hypothesis also includes the fact that all dominant mutations that cause AD are found in genes that are associated with APP metabolism (Haass 2004) whereas a mutation in the tau gene causes frontotemporal dementia (FTD) with parkinsonism, not AD (Hutton et al. 1998, Poorkaj et al. 1998, Spillantini et al. 1998). Moreover, in one FAD patient, amyloid pathology was shown to appear before NFTs (Smith et al. 2001). Patients with the tau mutation display severe NFT pathology and cognitive impairment, but do not exhibit amyloid plaques. These findings indicate that pathological changes in tau are not alone sufficient enough to provoke amyloid pathology. Furthermore, PS1, which is linked to amyloid pathology, may also protect tau from hyperphosphorylation, and therefore PS1 mutations may promote AD pathology also through tau (Baki et al. 2004). A PS1 mutation can also cause tauopathy without the presence of amyloid plaques (Dermaut et al. 2004). Another serious argument against the amyloid cascade hypothesis is the classical staging of AD neuropathology depicted by Braak & Braak, which states that NFT pathology precedes cortical amyloid pathology and the extent of NFT pathology correlates better with the gradual cognitive impairment (Braak and Braak 1991). However, NFTs may develop as part of the normal aging since they are found in all aged individuals regardless of their cognitive status (Price and Morris 1999). However, not only NFTs but also amyloid pathology can be detected in many asymptomatic elderly subjects (Bennett et al. 2006, Galvin et al. 2005, Neuropathology Group. Medical Research Council Cognitive Function and Aging Study 2001).

2.1.2 Clinical symptoms

When AD was first described, it was thought to be a rare condition that affected only a small number of relatively young patients. The cognitive impairment of the elderly was considered to be part of the normal aging process, caused by atherosclerosis of brain arteries. Later on, the neuropathological studies changed that view by revealing that AD associated pathology was a common feature in the elderly (Tomlinson et al. 1968). Although the onset of dementia varied greatly, the early onset and late onset dementia were considered to represent the same disease with uniform pathology.

From the diagnostic perspective this classification presents some difficulties. In early onset AD, the symptoms appear at an early age and usually progress quickly. This form of the disease is relatively easy to diagnose, because the symptoms are so distinct from most other diseases occurring at that age. However, in late onset AD, the symptoms develop later and progression is much slower. The pathological process of AD may begin as much as two decades before the clinical symptoms. During this preclinical period, it is not possible to detect patients who have AD pathology in their brain even with the most sophisticated neuropsychological tests (Driscoll et al. 2006). Even when the first cognitive symptoms occur, detecting the patients who have these symptoms due to AD is difficult because of the very insidious onset of symptoms. Similar symptoms may also be evoked by other causes such as depression or they can be regarded as normal cognitive changes associated with aging.

The salient feature of AD is the impairment in episodic memory, which is usually the first clinically evident symptom. Memory impairment is often the first clinical symptom because the AD pathology first occurs in MTL structures; a region that is essential for memory (Squire and Zola-Morgan 1991). However, a slight decline in memory occurs frequently as a part of normal aging. Later on in AD, patients exhibit impairment in other cognitive domains, for example executive function. This stage is often called "preclinical" AD in the literature, but it is highly questionable whether this term should be used, because these patients clearly have symptoms of the disease, although their symptoms are not severe enough to fulfil the diagnostic criteria for AD. During this earliest stage of the disease, these deficits do not prevent patients from carrying out normal activities of daily living, and therefore these patients cannot be yet diagnosed as having dementia. In clinically overt AD, patients exhibit severe and progressive deficits in other cognitive domains.

2.1.3 Concept of mild cognitive impairment

The belief that the cognitive symptoms of developing AD occur before the onset of clinical AD criteria fulfilling disease inspired researchers to try to find methods to discriminate these patients from healthy individuals. The starting point for most of the studies was memory impairment, because this is often considered as the first clinical sign of AD. However, memory impairment was not sufficient to be used alone, because it is associated with normal aging and other etiologies as well. Therefore more extensive criteria that aimed for more accurate distinction of AD patients were developed.(Table 1.) Several different criteria were introduced, but the fundamental tenet was that the patient would exhibit impairment beyond normal age related process but nonetheless would not be considered as being demented.

Table 1. Clinical criteria proposed for diagnosis of cognitive impairment

CIND	Objective impairment in one or more cognitive domains No dementia
AAMI	Subjective memory impairment Objective memory impairment compared to normative values
AACD	Subjective memory impairment Objective memory impairment compared to age-adjusted normative values
CDR 0.5	Subjective memory impairment CDR score 0.5
Amnestic MCI	Subjective memory impairment Normal activities of daily living Normal general cognitive functioning Objective memory impairment compared to age-adjusted normative values No dementia

CIND, cognitive impairment, no dementia; AAMI, age-associated memory impairment; AACD, aging-associated cognitive decline; CDR, clinical dementia rating; MCI, mild cognitive impairment.

2.2 MILD COGNITIVE IMPAIRMENT AND EARLY DIAGNOSIS OF AD

2.2.1 Mild cognitive impairment is a heterogeneous disorder

Initially, the term MCI was considered as a transitional state between cognitively healthy and clinically diagnosable AD. Currently, this view has broadened to encompass a state in which a patient exhibits cognitive impairment that is measurable in some objective way but the patient is not demented. One of the most extensively used set of criteria is based on that proposed by Petersen and co-workers (Petersen et al. 2001). The criteria divide the clinical presentation of MCI into four categories; MCI amnestic, MCI amnestic multiple domains, MCI single non-memory domain and MCI multiple non-memory domain categories. The criteria also acknowledge other possible causes of MCI than AD. Since AD is the most common dementing disease, it is also the most common etiology of MCI, either alone or as a concomitant pathology. AD is associated especially with amnestic MCI. It must be noted though that many patients with MCI do not have any underlying progressive disease.

Another popular definition of MCI is simply using the CDR score of 0.5 as a clinical criterion for MCI (Morris et al. 2001). The main outcome of these patients is AD, but also vascular dementia (VaD) and dementia with Lewy bodies (DLB) pathologies may develop. In many studies, this criterion for MCI is divided into domain specific subgroup depending on what cognitive domain is affected, for example amnestic or executive subtype. The problem with this definition is that the group of patients with a CDR score of 0.5 include both patients with MCI and patients with very mild dementia.

Cognitive impairment before the onset of clinically overt dementia is associated with other dementing diseases in addition to AD. The progression to other dementias was suggested to be associated with a decline in non-memory domains (Petersen et al. 2001). Indeed, a recent study reported that all of the MCI patients who progressed to FTD exhibited a decline in a single non-memory domain (Yaffe et al. 2006). Furthermore, another study reported that behavioural and affective symptoms together with executive dysfunction without decline in memory are associated with the development of FTD (de Mendonca et al. 2004). Vascular cognitive impairment (VCI) is proposed to be a prodromal condition of VaD (Bowler 2005). However, VCI patients include a very heterogeneous group of patients who progress to AD almost as often as to VaD and on the other hand, patients who fulfil the more general criteria for MCI often develop VaD frequently (Meyer et al. 2002b, Wentzel et al. 2001). A recent

study showed that both amnestic and non-amnestic MCI patients frequently develop dementia and also amnestic MCI patients developed VaD and DLB (Fischer et al. 2007). Furthermore, a neuropathological assessment of amnestic MCI patients who progressed to clinical dementia revealed that cognitive features cannot predict the development of AD associated neuropathology and most patients exhibit two or more different pathological features concomitantly (Jicha et al. 2006).

No matter what definition is used at the present, those patients diagnosed as having MCI include also patients who have no progressive neurodegenerative disorder. Psychiatric disorders, especially depression, may cause a cognitive decline without any progression to dementia (Ganguli et al. 2006). Depression is also an important condition to consider in the differential diagnosis of AD. Furthermore, anticholinergic drugs could contribute to cognitive impairment to such an extent that the patient may be diagnosed as having MCI (Ancelin et al. 2006). Therefore, it is not surprising that the group of patients diagnosed as having MCI will include some patients who eventually develop dementia, some who remain cognitively stable at MCI stage and even some who improve to become cognitively normal.

2.2.2 Pathological findings

The neuropathology of AD develops slowly during a long preclinical phase of the disease. Gradually the amount of neuropathology reaches a threshold to evoke cognitive symptoms that fulfil the clinical criteria for AD. Therefore it would be logical to presume that patients with MCI would display a substantial amount of AD associated neuropathology in their brain, though less than that found in patients with AD. Indeed, patients with MCI display marked AD neuropathology and its degree lies between the situation in cognitively healthy and AD patients with both CERAD and Braak staging (Bennett et al. 2005, Petersen et al. 2006). The NFT pathology of AD develops in a highly hierarchical sequential manner (Braak and Braak 1991). Consistent with this model, MCI patients have elevated number of NFTs in MTL structures when compared to healthy controls (Guillozet et al. 2003, Markesbery et al. 2006). The number of NFTs in MTL correlates with memory function (Guillozet et al. 2003). Furthermore, the early AD patients have an elevated number of NFTs in MTL and other brain areas as well (Markesbery et al. 2006). The extent of amyloid pathology falls also between the healthy controls and AD patients (Bennett et al. 2005, Markesbery et al. 2006). However, the number of NFTs correlate better with dementia severity, the number of amyloid plaques do

not display such a clear correlation (Berg et al. 1998). Since AD associated neuropathology is so prominent in MCI patients, some investigators have proposed that MCI is in fact a preclinical or early phase of AD (Markesbery et al. 2006, Morris et al. 2001). The picture is not quite so straightforward since both plaques and tangles are consistently found in the brains of cognitively healthy subjects, (Price and Morris 1999) even to an extend that should they have been examined simply in histopathological terms, the individuals would have been described as exhibiting AD pathology (Neuropathology Group. Medical Research Council Cognitive Function and Aging Study 2001).

2.2.3 Neuropsychological findings

Since memory impairment is a key feature in the most widely used MCI criteria, it has also been the most extensively studied cognitive domain in MCI (Petersen et al. 2001). Many recently published, longitudinal studies have used the earlier criteria in which a memory complaint was required for MCI diagnosis and impairment in other domains was prohibited (Petersen et al. 1999). However, several other cognitive domains have been studied, for example executive function, attention, praxis and language and visuospatial skills.

Although memory impairment is the most widely studied feature of MCI, isolated amnestic MCI is rare (Rasquin et al. 2005, Ribeiro et al. 2006, Ritchie et al. 2001, Tabert et al. 2006). In one study that included a consecutive sample of MCI patients, only 1.8% of the patients exhibited an isolated amnestic deficit. A total of 64.2% had multiple domains impaired and 17.0% of the MCI patients had a single non-memory domain impairment (Nordlund et al. 2005). In another study, 41.5% of the MCI patients in a memory clinic cohort did not have memory impairment (Rasquin et al. 2005). Even when relatively strict criteria for amnestic MCI are used, patients may also exhibit impairment in other cognitive domains if a detailed assessment of these other cognitive domains is performed (Kramer et al. 2006).

Many MCI patients have impairment in executive functions on conjunction with memory impairment (Lopez et al. 2006, Tabert et al. 2006) but executive dysfunction occurs also in patients without memory impairment (Lopez et al. 2006). A slight executive dysfunction may appear before any disturbances in functions of activities of daily living (Ready et al. 2003). Impairment in executive functions and language skills in MCI patients may be even more common than memory impairment (Nordlund et al. 2005). These findings challenge the

hypothesis that an isolated memory deficit is the first symptom of developing dementia and the symptoms then extend to other cognitive domains only with disease progression (Ribeiro et al. 2006).

2.2.4 Other features in mild cognitive impairment

Neuropsychiatric symptoms are relatively common in MCI patients, as many as 59% of patients have at least one neuropsychiatric symptom and up to 36% have three or more symptoms (Feldman et al. 2004). The most common symptoms noted in that study were depression, irritability, anxiety, agitation and apathy and the appearance of neuropsychiatric symptoms was associated with more severe cognitive impairment (Feldman et al. 2004). Another study reported that MCI patients had increased agitation, dysphoria, anxiety, apathy and irritability when compared to healthy controls. AD patients had similar symptoms, only the extent of delusional behaviour was further increased in AD when compared to MCI patients (Hwang et al. 2004). On the contrary, a large population-based study found that even though MCI patients have an elevated number of neuropsychiatric symptoms when compared to healthy controls, the AD patients exhibit still significantly more symptoms than MCI patients (Lyketsos et al. 2002). Nonetheless, neuropsychiatric symptoms, especially mood disturbances are common in MCI patients and are detrimental to the well-being of both patients and their families.

Although the decline in activities of daily living is restricted in the MCI criteria, a slight decline in functional capabilities is common in MCI patients (Farias et al. 2006). Those patients with a slight impairment in activities of daily living progress to dementia at a higher rate than patients with no functional decline and inclusion of decline in complex activities of daily living in MCI criteria might improve the sensitivity for development dementia (Peres et al. 2006). Motor dysfunction, particularly the parkinsonian signs, has also been associated with MCI and developing dementia (Aggarwal et al. 2006, Boyle et al. 2005).

2.2.5 Epidemiology and prognosis

Given the heterogeneity of the different criteria for MCI, it is not surprising that the incidence and prevalence rates of MCI in different studies vary greatly. The incidence, prevalence and progression rates to dementia are generally higher in clinic based studies than in population

based studies. When the cognitive impairment is defined according to broad criteria, the prevalence in the elderly population is high. The prevalence rate for age-associated cognitive decline (AACD) varies between 19.8% to 26.6% (Hanninen et al. 1996, Ritchie et al. 2001), but prevalence rates as low as 3.1% have been reported (Kumar et al. 2005). The prevalence of age-associated memory impairment (AAMI) has been reported to be 29% to 39% (Goldman and Morris 2001, Koivisto et al. 1995) or as low as 1.0% (Kumar et al. 2005). The prevalence of cognitive impairment no dementia (CIND) is close to the prevalence of AACD and AAMI, 16.8% (Graham et al. 1997).

The prevalence for MCI, defined with the Petersen criteria (Petersen et al. 1999, Petersen et al. 2001) is generally lower but also varies in the different studies. One of the reasons for these varying results is that even though the Petersen criteria are used, there have been numerous small modifications to the criteria between the studies. The prevalence and incidence rates vary also between different populations. In the population-based studies the prevalence rates vary between 2.8% and up to 28.3% (Hanninen et al. 2002, Larrieu et al. 2002, Lopez et al. 2003a, Manly et al. 2005). The prevalence of amnestic MCI is lower than the prevalence of MCI that also permits inclusion of other cognitive deficits. When both criteria for MCI were assessed in the same population, the prevalence for amnestic MCI was 6% and for the multiple cognitive deficits MCI 16% (Lopez et al. 2003a). In a Canadian population, the prevalence for amnestic MCI was only 1.0% (Fisk et al. 2003). The combination of memory impairment with impairment in another cognitive domain seems to be the most prevalent MCI type (Lopez et al. 2006, Ribeiro et al. 2006). The reported incidence rates in turn are between 9.9/1 000 and 25.9/1 000 person years among the non-demented elderly (Larrieu et al. 2002, Solfrizzi et al. 2004, Tervo et al. 2004).

The prognosis of MCI is heterogeneous possibly due to differences in etiology, studied population and inclusion criteria. Although MCI is viewed as the transitional state between the healthy aging and clinically overt dementia, i.e. an indication of a dementing disease in progress, as many as 40% of MCI patients revert to normal (Larrieu et al. 2002). Some MCI patients remain stable and are still classified as MCI at the follow-up as well. The most prevalent dementing disease as the outcome of progressive MCI is AD. Clinic based studies usually report higher conversion rates than the population based studies. The clinic based studies generally report conversion rates of 10-15% per year (Bozoki et al. 2001, Petersen et al. 1999, Rasquin et al. 2005). Conversion rates in population based studies are usually about

2-10% (Fisk et al. 2003, Larrieu et al. 2002, Solfrizzi et al. 2004). The reasons for this might be that the clinic based studies include more selected samples and possibly patients with slightly more advanced disease (Arnaiz et al. 2004). In a clinic based study that included only patients who were referred to the clinic by primary care physician after at least a three month history of memory complaint, the conversion rate was a high as 41% after one year and 64% after two year follow-up (Geslani et al. 2005). Furthermore, population based studies may include patients with reversible causes for cognitive impairment, for example anticholinergic drug use (Ancelin et al. 2006). The type of cognitive impairment also affects conversion rate. In a clinic based study that included patients with isolated amnestic MCI symptoms, the yearly conversion rate was 21.4% which is higher than that found in most clinic based studies (Perri et al. 2007). It seems that those MCI patients who have underlying progressing disease tend to convert to dementia within a few years time because the yearly conversion rates decrease in longer follow up from 10.8% in the first year to a mere 2.5% in the fifth and following years (Visser et al. 2006).

The risk factors for MCI are essentially similar to those for AD. The most important risk factor is increasing age. The strongest genetic risk factor for MCI and progression of MCI to dementia is the presence of at least one ApoE 4 allele (Hsiung et al. 2004, Manly et al. 2005, Petersen et al. 1995, Tervo et al. 2004). The ApoE 4 allele is especially detrimental to episodic memory, but it is also associated with accelerated decline also in other cognitive domains in the follow-up (Wilson et al. 2002). People with lower levels of formal education are at higher risk for MCI (Kivipelto et al. 2001, Lopez et al. 2003b, Manly et al. 2005, Tervo et al. 2004). Vascular risk factors, cardiovascular disease and elevated blood pressure, increase the risk of MCI and conversion to AD (Lopez et al. 2003b, Ravaglia et al. 2006, Solfrizzi et al. 2004, Tervo et al. 2004). An elevated blood cholesterol at midlife results in increased susceptibility for MCI later in life. Subjects with elevated blood pressure and high blood cholesterol at midlife are 2.7 times more likely to develop MCI two decades later than those subjects with normal values (Kivipelto et al. 2001). Cerebrovascular disease determined by MRI-identified infarcts also increases MCI risk (Lopez et al. 2003b). However, the association between the hypertension and MCI has not been found is other studies (Manly et al. 2005). Nonetheless, vascular risk factors seem to be associated with the development of MCI at least in some populations. Whether this association is causative is yet to be determined. The use of anticholinergic drugs may contribute to the cognitive impairment, such that after cessation of drug use the cognitive status returns to normal.

2.2.6 Towards a consensus - current concept of mild cognitive impairment

The heterogeneity of MCI criteria, epidemiology, prognosis and clinical outcome has raised some inevitable questions. First, is the syndrome currently called MCI in reality a uniform clinical entity? The etiology has been shown to be caused by several different pathological conditions or be even iatrogenic. Secondly, if the concept of MCI is accurate enough to identify patients with incipient AD, should the AD diagnosis itself be modified so that it includes these currently so called preclinical AD patients? Many researchers already consider MCI as early AD.

International Working Group on Mild Cognitive Impairment presented recommendations for general criteria for MCI after a symposium attended by specialists in different fields of dementia research (Winblad et al. 2004). Figure 1 represents the stepwise diagnostic process of MCI recommended by the group. The main difference with the former criteria is that there must be evidence of decline over time, the cognition of the patients is not compared to age adjusted normative values only. Also the MCI Working Group of the European Consortium on Alzheimer's Disease has recently proposed a new three stage diagnostic procedure for MCI (Portet et al. 2006). These criteria are essentially the same as those in the previous consensus report. In brief, to receive a MCI diagnosis, the patient needs to have a cognitive complaint, not necessarily a memory complaint, expressed by either the patient or a relative. There needs to be decline in cognitive functioning within the past year and the cognitive impairment should be corroborated by objective testing. Slight impairment in complex tasks of daily living are allowed but dementia must be excluded. The MCI subtype is then determined and additional examinations are initiated for detecting the underlying pathology.

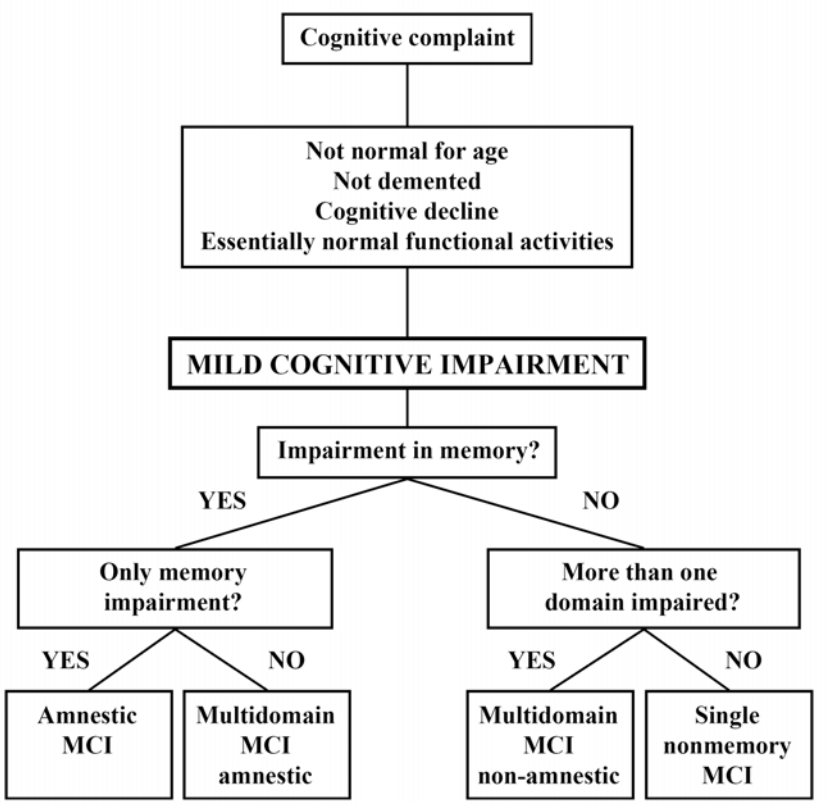

Figure 1. The recommendations of diagnostic classification process for MCI patients from the International Working Group on Mild Cognitive Impairment. Adapted from Winblad et al. 2004.

2.3 DEVELOPMENT OF DIAGNOSTIC TOOLS FOR EARLY DIAGNOSIS OF ALZHEIMER'S DISEASE

2.3.1 Search for a biomarker of early AD

When the diagnosis of AD is possible using current methods, the disease process is already far advanced. Even the most sophisticated neuropsychological testing and clinical examination cannot reliably pinpoint those MCI patients who will eventually develop AD, much less those subjects who are still cognitively healthy but already have on-going neuropathology processes in the brain (Driscoll et al. 2006). Also, current methods are not sensitive enough to observe the possible, disease-modifying effects of different treatments for AD, either current or still experimental therapies. Therefore, there is a dire need for an

objective biomarker for AD.

The Working group on "Molecular and Biochemical Markers of Alzheimer's Disease" stated in 1998 that a biomarker should be able to detect a fundamental feature of AD neuropathology, be validated in neuropathologically confirmed AD cases, and it should have sensitivity and specificity of >80%. It should also be precise, reliable, non-invasive, simple to perform and inexpensive (Consensus report of the Working Group on: "Molecular and Biochemical Markers of Alzheimer's Disease". The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group. 1998). Since AD has a long preclinical stage of the disease, the biomarker should also be predictive. In AD, naturally, the biomarker should be relevantly linked to either amyloid- or tau pathology.

There are two approaches for searching possible biomarkers. The discovery type of approach searches new biomarkers from plasma or CSF from a limited number of cases using methods that analyse many different molecules simultaneously, for example proteomics. When it is believed that there is a molecule that differs between the AD patients and controls, this molecule is identified and then examined further in larger study population. Another approach is the hypothesis driven search for a biomarker. Some already known feature of AD associated pathology is studied in a group of AD patients and healthy controls, and the accuracy of this feature in distinguishing patients with AD is calculated. For example atrophy of MTL structures is a well known characteristic of AD, and MRI imaging of atrophy has been assessed as a potential biomarker.

The key requirement for an AD biomarker is that it should be validated in neuropathologically confirmed AD cases. Neuropathological confirmation could be reached either by biopsy of the brain, which is not in practice in AD diagnostics, or by neuropathological examination after death. Therefore the majority of the studies on biomarkers are conducted on clinical cohorts and this leads to some problems. The clinical diagnosis of AD reaches relatively high accuracy in experienced clinical units, but nonetheless, sensitivity remains at about 85% of the AD cases. Furthermore, the accuracy of clinical AD diagnosis is poor especially in the early stages of the disease, so the follow-up should be long enough to detect true AD patients. This problem is of course even more severe when studying MCI patients, who do not fulfil the criteria for even possible AD. Therefore, when studying AD biomarkers for prediction of

AD in MCI patients, the study population needs to be large and the follow-up has to last several years at the minimum. Another significant problem is that some cognitively healthy subjects exhibit substantial AD type neuropathology (Price and Morris 1999). On the other hand, some patients with severe cognitive impairment display very little neuropathology. It is also common that patients with other dementias, for example VaD or DLB, have concomitant AD neuropathology or AD patients have vascular or LB pathology (Neuropathology Group. Medical Research Council Cognitive Function and Aging Study 2001). The presence of different pathologies cannot be detected by the current clinical methods.

2.3.2 Imaging

At present in AD diagnosis, the brain imaging is used mainly for the exclusion of other possible causes of dementia, for example vascular disease. In MRI and CT it is also possible to see general brain atrophy in AD patients, but this atrophy is in no means either sensitive or specific to AD. Since imaging techniques are non-invasive, there has been great interest in developing imaging based methods on an early diagnosis of AD.

2.3.2.1 Magnetic resonance imaging

In addition to general brain atrophy, ongoing neurodegeneration of AD causes atrophy of MTL structures, especially HC and EC. The MTL atrophy occurs early in development of AD, which is in accordance with the Braak model of AD pathology (Braak and Braak 1991). This atrophy can be seen in CT, but MRI has proved to be better and more sensitive especially in detecting slight changes. Since the AD neuropathology begins from MTL, the HC and EC atrophy has been studied extensively in the hope of finding a possible preclinical imaging marker for AD. Several studies have shown that EC and HC atrophy is a typical feature of AD (Bobinski et al. 2000, Jack et al. 2002, Pennanen et al. 2004) and it is associated with decreased number of neurones of the region (Bobinski et al. 2000, Simic et al. 1997). However, MTL atrophy is not specific to AD but it also occurs in other neurodegenerative diseases (Jack et al. 2002). In MCI patients, the atrophy seems to lie between the healthy subjects and AD patients (Pennanen et al. 2004). Individual variability is large, but there is evidence that MTL atrophy may be useful in detecting those MCI patients who are at greater risk for development of AD. Since EC is affected before HC by AD pathology, especially during MCI stage of the disease, measurement of EC and HC separately may have better

diagnostic value than evaluating overall MTL atrophy (Pennanen et al. 2004). Nonetheless it is possible that because the EC is a rather small structure, the technical and anatomical limitations may nullify this theoretical benefit (Xu et al. 2000).

A more practical clinical way of assessing MTL atrophy is to use a qualitative approach, because it enables grading of MTL atrophy from one imaging plane. The grading system can be easily learned, and acceptable inter-rater accuracy can be achieved (Scheltens et al. 1992). This type of rating can distinguish AD patients from healthy controls with an accuracy of 96% when used in a clinically rational context (Wahlund et al. 2000). It can also be used as an aid to predict AD in MCI patients (Bouwman et al. 2006, Korf et al. 2004). Volumetric analysis is more detailed and may have better predictive accuracy. However, volumetric analysis is time consuming and laborious and it places great demands on the skills of the analyst. Moreover, HC and EC atrophy may not provide significant added value for a diagnosis between AD and other dementias when compared to neuropsychological testing. In a study comparing the diagnostic value of mini mental state examination (MMSE) and MTL atrophy, the overall accuracy of MMSE alone was 88% whereas MMSE in conjunction with volumetric or visual MTL atrophy rating was only 90% (Wahlund et al. 2000). Another study examined the predictive value of neuropsychiatric assessment and MTL atrophy for development of dementia and found that the accuracy of MMSE together with two other neuropsychiatric tests was 79.6% and accuracy of neuropsychiatric tests together with MTL atrophy 86.8% (Devanand et al. 2007). Therefore, at least for the time being, inclusion of volumetric MRI is not appropriate as a component of the clinical work-up. Functional MRI has also been assessed as a possible diagnostic aid for early AD, but the results are varying and the method is very laborious for both patient and analyst.

2.3.2.2 Functional imaging

Positron emission tomography (PET) and single photon emission computed tomography (SPECT) have been studied as possible biomarkers for AD. The most commonly used method in PET is the examination of regional glucose metabolism of different brain areas by using radio-labelled [18F] fluorodeoxyglucose. SPECT is used for imaging of regional blood flow. In neuropathologically confirmed patients, the hypoperfusion in parietal and temporal lobes yielded a sensitivity of 89% and a specificity of 80% against healthy controls and patients with other dementias (Jobst et al. 1998). Another study showed that the severity of

hypoperfusion was associated with the severity of cognitive impairment in AD patients (Waldemar et al. 1994). Temporal lobe hypoperfusion occurs already in patients with amnestic symptoms and hypoperfusion of posterior cingulate cortex has been reported to differentiate the MCI patients with a cognitive decline at the five year follow-up from stable MCI patients with 79% sensitivity and 67% specificity (Hogh et al. 2004, Johnson et al. 2007). The accuracy of PET imaging seems to be better than the accuracy of SPECT (Mosconi 2005). The hypometabolism in MTL structures and posterior cingulate cortex has been shown to occur already in mild AD (Mosconi et al. 2007). Hypometabolism in parietal and temporal regions was claimed to differentiate AD patients from controls with 93% sensitivity and specificity and MCI patients who develop dementia from stable MCI patients with 93% sensitivity and 82% specificity (Anchisi et al. 2005, Herholz et al. 2002).

2.3.2.3 Molecular imaging

However, it is another PET based technique that has been raised the greatest hopes of improving AD diagnostics. Thioflavin compounds are traditionally used in histopathological examination of many tissues because they bind to the amyloids formed by different amyloidogenic proteins. In AD, the thioflavin compounds are routinely used for staining amyloid plaques that contain fibrillar Aβ. Pittsburgh Compound-B (PIB) is a thioflavin T derivative that can cross the blood brain barrier (BBB). In living AD and amnestic MCI patients, the PIB load as detected in PET is significantly higher than in healthy controls (Kemppainen et al. 2007, Klunk et al. 2004), and PIB binding correlates with the extent of brain atrophy (Archer et al. 2006). PIB binding also correlates with hypometabolism in temporal and parietal cortices of brain, although this association is lacking in frontal cortex (Edison et al. 2007). The distribution of PIB binding in brain is in accordance with the distribution of amyloid pathology found in autopsy studies on AD patients (Kemppainen et al. 2006). Furthermore, in a recent study, all of the examined AD patients had PIB positive scans. However, that study also reported that four of 12 frontotemporal lobar degeneration patients and one out eight controls displayed positive PIB binding (Rabinovici et al. 2007). It is possible that these false positives were subjects with underlying amyloid pathology, because no neuropathological validation was available. One obstacle to using PIB as a AD biomarker is that even though PIB binding accurately confirms the presence of amyloid, it seems that it cannot distinguish between the amyloid in plaques and as cerebral amyloid angiopathy in blood vessels (Bacskai et al. 2007). No large follow-up studies are available, but it does seem

plausible that PIB could be used as an imaging biomarker for amyloid pathology in AD and even for progressive MCI.

2.3.3 Laboratory tests for Alzheimer's disease

2.3.3.1 Plasma Aβ peptide

A biomarker that could be found in plasma would be ideal because a blood sample is easy to obtain and routinely used in clinical practice, minimally invasive and safe. The credibility of plasma biomarker is supported by the fact that the BBB damage is associated with AD pathology. Since APP and its metabolites, including Aβ peptide can be found in plasma, their feasibility for use in AD diagnosis has been studied. A large variety of other types of molecules have also been studied, but the results have been variable and at least for the time being, none of them have any validity in AD diagnosis (Irizarry 2004).

APP, PS1 and PS2 mutation carriers as well as patients with DS who possess three copies of the APP gene were shown to have elevated plasma Aβ42 levels when compared to control subjects (Mehta et al. 1998, Scheuner et al. 1996). Also AD patients were reported to have increased Aβ42 levels when compared to healthy controls (Younkin et al. 2000). Furthermore, AD patients and those patients who developed AD during the follow-up had increased Aβ42 levels when compared to controls (Mayeux et al. 2003). Another study reported elevated plasma Aβ40 but no difference in plasma Aβ42 levels between AD patients and controls (Mehta et al. 2000). In addition, in a third study, neither Aβ40 nor Aβ42 were different between AD patients and controls, but they correlated with age in each group (Fukumoto et al. 2003). The picture is as diverse in patients with MCI; Aβ42 levels have been shown to be increased in women with MCI when compared to controls or men with MCI (Assini et al. 2004) but in another study there was no difference between groups (Fukumoto et al. 2003). Other amyloid related molecules studied as potential biomarker include Aβ directed autoantibodies found in plasma and the ratio of platelet derived APP isoforms (Di Luca et al. 1998).

2.3.3.2 Cerebrospinal fluid amyloid beta peptide

CSF is in direct contact with brain and thus reflects brain associated biochemical events better

than any other biological fluid. Therefore CSF is a more likely source for finding a clinically usable biomarker than plasma. Aβ, tau and phospho-tau are linked to AD associated neuropathological changes, and they have been the most widely studied potential biomarkers for AD. Although most of the CSF is produced in the choroid plexus by capillary filtration, a substantial proportion is derived from extracellular fluid of brain parenchyma and about 20% of CSF proteins are synthesized in brain. Aβ peptide is secreted during physiological metabolism and it is a normal constituent of human CSF (Haass et al. 1992, Seubert et al. 1992). The first studies on total CSF Aβ as a biomarker for AD gave disappointing results. Studies showed that CSF total Aβ levels were decreased (Pirttila et al. 1994), increased (Nakamura et al. 1994) or not changed at all in AD patients (Motter et al. 1995, van Gool et al. 1995). After the discovery of the two main types of Aβ peptides in CSF, Aβ40 and Aβ42, it was found that CSF Aβ42 levels were consistently lower in AD (Motter et al. 1995). Subsequently it was established that not only CSF Aβ42 was lower in AD, but it also could distinguish patients with AD from healthy controls with reasonable accuracy, even in patients with mild AD (Blennow and Hampel 2003).

In MCI patients the results have been somewhat inconsistent. In a cross-sectional study, a small group of subjects with mild memory impairment exhibited low CSF Aβ42 levels (Hulstaert et al. 1999), whereas another study on a larger group of MCI patients reported significantly elevated Aβ42 levels in MCI patients when compared to healthy controls (Jensen et al. 1999). In a follow-up study on 19 MCI patients, the baseline levels of CSF Aβ42 were similar between the MCI group and the healthy control group, but when the MCI patients developed clinically overt AD, then the Aβ42 levels decreased (Maruyama et al. 2001).

There is huge interest in differentiating those MCI patients who will develop dementia from those who remain stable or even improve to cognitively healthy. One study included 15 cognitively healthy controls and 16 MCI patients who developed dementia within one year follow-up, and it was found that MCI patients had significantly lower CSF Aβ42 levels at the baseline (Andreasen et al. 1999). In another study, the baseline Aβ42 levels were lower in MCI patients who developed AD or had some other progressive cognitive impairment but did not develop dementia within an 18 month follow-up when compared to those MCI patients whose cognition remained stable (Riemenschneider et al. 2000). Furthermore, a study that included 52 MCI patients found that those 23 patients who developed probable AD during a

seven month follow-up had lower Aβ42 levels when compared to nonconverters (Hampel et al. 2004). However, in a study including 53 MCI patients, of whom 22 developed AD during an average 20 month follow-up, the baseline Aβ42 levels did not reveal any statistically significant difference between converters and non-converters (Zetterberg et al. 2003).

In studies on MCI patients, the follow-up period should be much longer than used in these studies, because the development of dementia in MCI patients may take several years. In a study with too short a follow-up time, the stable MCI group is likely to include patients who would develop dementia during longer follow-up. The results of studies with longer follow-up are starting to emerge. A large study with 137 MCI patients reported that those MCI patients who progress to dementia within an average of five years follow-up had decreased CSF Aβ42 levels at the baseline (Hansson et al. 2006). Therefore, the reduced Aβ levels seem to be evident even several years before the onset of dementia. Furthermore, CSF Aβ42 levels were shown to be lower in cognitively healthy subjects who developed dementia after three years follow-up (Skoog et al. 2003). The same group have extended their study and observed that decreased Aβ42 levels are associated with decreased MMSE score after eight years follow-up (Gustafson et al. 2006).

CSF Aβ levels are thought to be associated with the amyloid pathology of the brain, but few studies have compared CSF Aβ levels and brain pathology. One study correlated *post mortem* ventricular CSF Aβ42 levels to the amount of brain amyloid pathology and found that more pronounced amyloid pathology was associated with lower Aβ42 levels in CSF (Strozyk et al. 2003). Other studies have reported decreased CSF Aβ42 levels in neuropathologically confirmed AD patients, but did not report data on the quantitative amount of amyloid pathology (Clark et al. 2003, Engelborghs et al. 2007). The reasons for the decreased CSF Aβ42 levels in AD are not known yet, but it is possibly due decreased production resulting from cell death or abnormal clearance from brain to CSF as amyloid plaques sequester the Aβ peptides.

2.3.3.3 Cerebrospinal fluid tau

CSF tau has been consistently shown to be increased in AD when compared to healthy subjects relatively soon after the measurement of CSF tau became possible (Jensen et al.

1995, Vandermeeren et al. 1993, Vigo-Pelfrey et al. 1995). The association between AD and increased tau levels has been corroborated also in neuropathologically confirmed patients (Clark et al. 2003). These results have been replicated in several studies, and CSF tau was established as one of the most promising biomarkers for AD (Blennow and Hampel 2003). CSF tau levels were found to be increased even in patients with very mild AD (Kurz et al. 1998, Riemenschneider et al. 1996). In the cross-sectional setting, the patients with minimal memory impairment or MCI had higher CSF tau levels than the healthy controls (Hulstaert et al. 1999, Maruyama et al. 2001). Baseline tau levels were also associated with developing dementia in the follow-up (Andreasen et al. 1999, Maruyama et al. 2001, Riemenschneider et al. 2000, Zetterberg et al. 2003). There is evidence that in MCI patients, the CSF tau levels are increased in those patients who develop AD compared to those who develop some other form of dementia (Zetterberg et al. 2003). However, the association between increased tau and developing AD has not been confirmed in all studies (Buerger et al. 2002a).

Although there is a body of evidence that increased CSF tau levels are associated with AD, even during an early stage of the disease, problems in using tau as a biomarker soon emerged. An increased tau level was not specific to AD, but seemed to be associated with many neurodegenerative states. In a population based study, the patients with VaD or mixed AD and VaD had similar tau values to AD patients (Andreasen et al. 1998). Increased tau levels were also reported from patients suffering from DLB or FTD (Arai et al. 1997). Extremely high tau values are found in patients with Creutzfeldt-Jakob disease (CJD) (Otto et al. 1997) and patients with recent stroke (Hesse et al. 2001). Although some of these findings proved inconsistent, it became obvious that tau mirrors neuronal and axonal damage in general rather than the neurodegenerative process of AD specifically. Thereafter, the search for a reliable biomarker moved on to find a more AD specific form of tau.

Table 2. The characteristics of CSF Aβ42 and tau in predicting dementia in MCI patients

		Stable MCI Specificity	Progressive MCI Sensitivity	Overall Accuracy	Positive LR	Negative LR
Riemenschneider et al. 2002[1]	Aβ42 + tau	90% 9 / 10	90% 9 / 10	90% 18 / 20	9.00 (1.35-58.44)	0.11 (0.02-0.71)
Hampel et al. 2004[2]	Aβ42	57% 13 / 23	83% 24 / 29	71% 37 / 52	1.90 (1.61-3.12)	0.31 (0.13-0.73)
	Tau	48% 11 / 23	90% 26 / 29	71% 37 / 52	1.72 (1.14-2.59)	0.22 (0.07-0.69)
Maruyama et al. 2004[3]	Tau	87.5% 14 / 16	88% 36 / 41	88% 50 / 57	7.02 (1.91-25.81)	0.14 (0.06-0.32)
Hansson et al. 2006[4]	Aβ42 + tau	82% 46 / 56	95% 54 / 57	88% 100 / 113	5.31 (3.01-9.34)	0.06 (0.02-0.19)

The table contains all the studies that have provided sufficient information concerning stable and progressive MCI patients to make it possible to calculate diagnostic accuracy figures. Data presented as percentage and number of correctly allocated subjects in the group or as LR (95% CI). MCI, mild cognitive impairment; LR, likelihood ratio.

1. Endpoint Alzheimer's disease, cut-off: Aβ42 = 240 + 1.18 × tau

2. Endpoint Alzheimer's disease, cut-off: Aβ42 679 pg/ml and tau 479 pg/ml

3. Endpoint dementia, cut-off: tau 320 pg/ml

4. Endpoint Alzheimer's disease, cut-off: Aβ42530 pg/ml and tau 350 pg/ml

2.3.3.4 Cerebrospinal fluid phospho-tau

Phosphorylation of tau is a normal physiological event, and it may occur in several dozens of amino acid residues of tau. In AD, as well as in some other neurodegenerative diseases associated with tau pathology, tau is phosphorylated beyond the normal functional level. The result is hyperphosphorylated tau which impairs axonal transport and forms intracellular aggregates (Avila 2006). There are several assays for measurement of CSF phospho-tau, but the most widely utilized are the assays for phosphorylated at threonine 181 (phospho-tau181) and threonine 231 (phospho-tau231) both of which seem to perform equally well in discriminating AD patients from controls and patients with other neurological diseases (Hampel et al. 2004).

Measurement of phospho-tau instead of total tau especially improves the discrimination of AD patients from patients with DLB or FTD (Hampel et al. 2004, Sjogren et al. 2001). The

benefits of phospho-tau are also that unlike total tau, phospho-tau is not markedly increased in CJD (Buerger et al. 2006b, Riemenschneider et al. 2003) or after a stroke (Hesse et al. 2001). Elevated phospho-tau levels have been found in progressive MCI patients (Arai et al. 2000) and phospho-tau levels have been reported to display a correlation to annual points lost in MMSE (Buerger et al. 2002a, Buerger et al. 2002b).

2.3.3.5 Other possible CSF biomarkers

Numerous different proteins that are present in CSF have been studied as potential biomarkers for AD in the past decade. Many of the studied proteins are related to different aspects of AD pathology, for example the presence of glycosylated acetylcholinesterase may reflect the changes of cholinergic system in AD brain (Saez-Valero et al. 2000). Apart from tau proteins, other molecules that accompany neuronal cell death have been examined; one example of these is tissue transglutaminase (Bonelli et al. 2002). There are also studies into a large number of possible biomarkers for inflammation of AD brain (Jia et al. 2005). Molecules that reflect homeostasis of the brain have been proposed as representing possible biomarkers, these include 24S-hydroxycholesterol which is a brain specific metabolite of cholesterol (Leoni et al. 2006) and isoprostane which is a marker for oxidative damage (Montine et al. 2001). It is also possible that the most accurate diagnosis of early AD can be achieved by using a panel of several different biomarkers (Simonsen et al. 2007). Even though the above mentioned and numerous other molecules including neurofilament proteins, neuromodulin and neuronal thread protein have been studied (Blennow 2004), at the present, the only usable biomarkers are the CSF Aβ42, tau and phospho-tau levels in CSF.

3. AIMS OF THE STUDY

AD is a disease that has been suggested to develop slowly over a time range of two decades before the first symptoms make their appearance. Neuropathology starts to develop early during a presymptomatic period of the disease, and at the first symptomatic stage, the pathology is already advanced. This is a great obstacle in treating the disease because repairing the already existing damage is currently impossible. Previous studies have suggested that changes in CSF biomarkers Aβ42, tau and phospho-tau can be used as a diagnostic aid especially in clinically challenging conditions, for example in the differentiation between mild AD and depression.

At the onset of this study, little was known about these markers before the onset of clinical AD. Since AD neuropathology starts to develop decades before the appearance of symptoms, the original hypothesis was that AD associated changes in CSF biomarkers could be detected before these clinical signs of AD. The main aim of the study was to examine whether these changes would predict the development of AD in patients with MCI and whether there was an association between AD associated pathological changes in brain and the CSF biomarker levels.

The specific aims of the study were:

1. To study whether the CSF Aβ levels are related to amyloid pathology in the brain of the transgenic mouse model of AD.
2. To study whether the AD associated changes in CSF biomarker are detectable before the clinical diagnosis of AD.
3. To study whether these changes are predictive for AD when used as a diagnostic test.
4. To study the association between the CSF biomarkers and changes in medial temporal lobe structures.

4. MATERIALS AND METHODS

4.1 SUBJECTS

4.1.1 Study population

The study population was derived from two different sources. The majority, 107 of the subjects, were recruited from the neurological department of the Kuopio University Hospital to which they had been referred by their primary care physician due to neurological symptoms. The reasons for referral included headaches, dizziness and cognitive symptoms. Those patients who agreed to undergo lumbar puncture for research purposes were included in the study. The patients who were found to be neurologically intact or whose symptoms were caused by depression were designated as controls. The diagnosis of depression was based on the clinical examination and patients with depression were further referred to psychiatrist. The MCI patients included both amnestic MCI patients and patients with some other type of cognitive decline, for example executive dysfunction or impaired visuospatial or language skills. One subject with fibromyalgic pains, who entered the study as a control subject, was excluded due to receiving a diagnosis of AD ten years later. A total of 33 subjects included in the study were part of a large population-based follow-up study of cognitive impairment in the elderly population of Kuopio region. Those patients who agreed to undergo lumbar puncture for research purposes were included in the study. All subjects gave informed consent and the study was approved at the Kuopio University Hospital Ethical Committee.

In study **II** all of the MCI patients were analysed as a single group without any more detailed division into diagnostic groups. For study **III**, the MCI patients were divided into three different groups based on the MCI type: amnestic MCI (with or without any other cognitive impairment), executive MCI (with or without any other cognitive impairment, no amnestic symptoms) and other MCI (subjects with impairment in one or more cognitive domains other than memory or executive function). In study **III**, the control group was divided into patients with depression and other controls, since depression is one of the most important diseases to consider in the differential diagnosis of early AD. The study **IV** included all the MCI patients from the larger neuroimaging study (Pennanen et al. 2004) who had also agreed to a lumbar

puncture and therefore for whom biomarker data were available. All of these patients except one were also included in study **III**. In all of the studies **II-IV**, the MCI patients were further divided into those who remained cognitively stable and those who developed dementia during the follow-up.

Baseline examination of all subjects included a neurological and neuropsychological examination, imaging of the brain (mainly computed tomography), and laboratory tests for exclusion of the secondary causes of cognitive decline according to Finnish guidelines. The subjects from whom the follow-up information was available formed the final cohort of the study. The follow-up examinations were performed yearly for the population based group. The patients from the clinic group were either seen again at the clinic or the follow-up data were later derived from the medical charts of the patients.

4.1.2 Neuropsychological testing

The large variety of neuropsychological tests was used to assess impairment in different cognitive domains. In the clinical group, the tests that were used varied because the baseline examination of patients was conducted over a period of several years. However, all of the domains that were studied in the population based group were assessed also in the clinical group.

In the population based group, the following tests were used: *Memory:* Visual Reproduction Test (VR, immediate and delayed recall) from Wechsler Memory Scale (WMS) (Russel 1975), Logical Memory Test (LM, immediate and delayed recall) from Wechsler Memory Scale-Revised (WMS-R) (Wechsler 1987), Word List Recall (immediate and delayed recall) from the CERAD Neuropsychological Assessment Battery (Morris et al. 1989), Delayed Recall of the Constructional Praxis from CERAD (Morris et al. 1989); *Language:* Abbreviated (15 items) Boston Naming Test (Kaplan et al. 1991), vocabulary subtest from the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Wechsler 1981); *Attention and executive function:* Verbal Fluency Test (Borkowski et al. 1967, Butters et al. 1987), Trail Making Test (Reitan 1958) parts A and B; *Visuospatial skills:* Constructional Praxis from CERAD (Morris et al. 1989), Block Design from the WAIS-R (Wechsler 1981); *Global functioning:* Mini-Mental State Examination (Folstein et al. 1975) (MMSE), Clock Drawing Test. (Morris et al. 1989) Subjects performing below the age-adjusted norms in at least one

cognitive domain were diagnosed as having MCI if they also had a score of 0.5 on the CDR scale (Morris 1993). The assessment of CDR score was based on a non-structured clinical interview on patient and caregiver.

4.1.3 Diagnosis of dementia

The diagnosis of dementia was based on the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (American Psychiatric Association 1994) and AD was diagnosed using the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (McKhann et al. 1984). The diagnosis was made by consensus decision by two neurologists and two neuropsychologists. The diagnosis was made based on the clinical symptoms and neuropsychological test results. The diagnostic workup included the standard laboratory testing and imaging, mainly computed tomography, to exclude any other possible causes for dementia than AD. The diagnosis of dementia was done independently and blinded to the CSF biomarker data and the ApoE genotype.

4.2 TRANSGENIC MICE

4.2.1 Transgenic mice

Two different doubly transgenic mouse lines were used in the study. Both lines carry an APP695 gene that includes the human Aβ domain with mutations K595N and M596L that are linked to Swedish familial AD pedigrees (APPswe). The first mouse line also carries a human PS1 gene with AD linked mutation A246E (APPswe/PS1 mice) (Borchelt et al. 1997). The second line carries a PS1-dE9 mutation (APPswe/PS1dE9) (Jankowsky et al. 2004).

4.2.2 Surgical procedures

The sampling method for mouse CSF was adapted from a previously described method (Meyding-Lamade et al. 1996). CSF was taken from 22 APPswe/PS1 mice at the age of 5 and 7 months and from 39 mice at the age of 8, 10 and 13 months. From 36 APPswe/PS1dE9 mice, the CSF sample was taken at the age of 6 and 9 months. CSF was collected by penetrating the dura in the cisterna magna with a 6-cm-long glass capillary that had a tip with an outer diameter of 0.5 mm. The sample was immediately transferred to a polypropylene

tube and stored at -70°C until analyses. The average collected volume of CSF was approximately 7 µl.

After the last round of CSF sampling, the mice were transcardially perfused with 50 ml heparinized ice-cold 0.9% saline (10 ml/min), and the brains were rapidly removed. One hemibrain was immediately immersed in 4% paraformaldehyde in 0.1 M Na-phosphate buffer for 4 h and then transferred overnight to 30% sucrose solution. These samples were then stored in a cryoprotectant at -20°C until histological analyses. The other hemibrain was dissected on ice into selected brain areas (including HC and cerebellum) that were stored at -70°C until ELISA analyses.

4.2.3 Sample preparation for ELISA

The analysis focused on the HC which was evaluated from all mice that were included in the study. The cerebellar Aβ42 was also studied from a subset of mice because the cisterna magna lies next to the cerebellum, and therefore it is possible that CSF Aβ42 originates from cerebellum instead of the main plaque forming areas of the brain, including HC. The tissue sample was first homogenized in phosphate buffered saline, pH 7.2, which contained a mixture of protease inhibitors (Complete^{TM}, Boehringen Mannheim, Germany). Tissue homogenates were then centrifuged at 218 000 × g for 2 h at +4°C. The supernatants that contained the soluble Aβ42 were collected and stored at -70°C until analyses. The remaining pellet containing the insoluble Aβ42 was further homogenized in guanidine buffer (5.0 M guanidine-HCl in 50 mM Tris-HCl, pH 8.0), incubated at room temperature for 3 h, centrifuged, and diluted to reduce the guanidine concentration to 0.5 M.

4.2.4 Histology

The brain was cut into 30 µm coronal sections, which were stained with W02 antibody (ZMBH, Heidelberg, Germany) that recognizes the human Aβ as described earlier (van Groen et al. 2003). Three stained sections through the HC were digitized using a Nikon Coolpix 990 camera, and the images were converted to grey scale using the Photoshop 5 program. The percentage of area covered by the reaction product to Aβ (i.e. Aβ load) was measured using the ScionImage (NIH) program.

4.3 CSF BIOMARKER ANALYSES

4.3.1 Aβ42

Human CSF Aβ42 levels were measured by using an ELISA kit (Innogenetics, Ghent, Belgium). All analyses were done in duplicate. The mouse CSF Aβ42 levels were measured by using the High Sensitivity method provided in the same kit. Due to the small amount of available sample, the analyses were done without duplicates. The brain Aβ42 was measured by the High Sensitivity method according to the protocol, except that 0.5 M guanidine was added to the kit's standards when measuring the insoluble Aβ42 from brain tissue samples. This was done because guanidine significantly decreases the strength of the signal in ELISA and if the guanidine was missing from standards the results would have been erroneously low. The brain Aβ42 from the 7 month old mice was measured by using a Signal Select β-amyloid ELISA kit (BioSource International Inc.).

4.3.2 Tau and phospho-tau

CSF tau levels were measured by using an ELISA kit (Innogenetics, Ghent, Belgium). The CSF phospho-tau levels were measured by using an ELISA kit that measures specifically the Tau phosphorylated at residue 181. All analyses were made in duplicate.

4.4 APOE GENOTYPING

The ApoE genotype was determined by using a PCR based method (Tsukamoto et al. 1993). For the statistical analyses, the subjects were classified according their ApoE genotype; either by the presence (ApoE 4 positive) or absence (ApoE 4 negative) of ApoE 4 or the number of possessed ApoE 4 alleles (none, one or two ApoE 4 alleles).

4.5 MAGNETIC RESONANCE IMAGING

All subjects in study **IV** underwent a high resolution MRI in the Kuopio University Hospital. The images were acquired using a 1.5 Tesla Vision (Siemens, Erlangen, Germany), with a 3D magnetization prepared rapid acquisition gradient echo sequence (for the patients scanned in 1998/1999, the parameters were: TR (time of repetition) = 9.7 ms, TE (time of echo) = 4 ms, matrix 256×256, 1 acquisition and in plane resolution = 0.98 mm, while for the patients

scanned in 1999-2001 the parameters were: TR = 13.5, TE = 7, matrix 256x256, 1 acquisition and in plane resolution = 0.94 mm). Standard neuroanatomical landmarks were used to correct for possible deviations in any of the orthogonal planes and the scans were reconstructed into 2.0 mm thick contiguous coronal slices, oriented perpendicular to the intercommissural line. The hippocampi and EC were manually traced by a single tracer (C.P.), using custom-made software for a standard Siemens work console (Pennanen et al. 2004). The boundaries of the regions of interest were outlined using a trackball driven cursor, always proceeding in an anterior to posterior direction. The coronal intracranial area at the level of the anterior commissure was measured and used for the normalization of the volumetric data according to the formula: (volume/intracranial area) x 100. The intraclass correlation coefficients for intrarater reliability were 0.96 for the HC and 0.95 for the EC measured from 10 subjects. All analyses were done blinded to the clinical data (Pennanen et al. 2004).

4.6 STATISTICAL ANALYSIS

All statistical analyses were performed by using SPSS software for Windows (SPSS Inc. Chicago, IL). The comparisons between the different groups of subjects were done by ANOVA with appropriate *post hoc* corrections. When the assumptions for normality were not met, the nonparametric tests were used. For the categorical data, the comparisons between different groups were made with Chi-square tests. The correlations between different variables were calculated by Pearson's correlation test or by Spearman's correlation test. The best cut-off values for development of dementia of different measures were determined by receiver operator characteristics (ROC) analysis. The odds ratios for different factors that might contribute to the conversion of MCI to dementia were determined by logistic regression analysis, so that only the patients with MCI at the baseline were included. Cox regression analysis was used for survival analyses.

4.7 ETHICAL CONSIDERATIONS

Elderly patients with cognitive impairment are a very special group to consider in clinical studies. Additional caution needs to be used when informed consent is obtained. All of the patients who entered the study were only slightly impaired at the time of inclusion into the study and thus capable of providing informed consent. Since CSF biomarkers and ApoE allele

analysis are not currently used in routine diagnostics, the results of these analyses were not revealed to the patients.

Lumbar puncture is an invasive method that may be uncomfortable for the patient and sometimes has side effects. However, in a study on 342 subjects, it was shown to be a safe procedure, and even the post puncture headache, the most frequent side effect, was rare especially in the patients with AD and MCI (Peskind et al. 2005). On the other hand, the dire need for the development of methods for earlier diagnosis of AD is increasing as the treatment possibilities progress. Since CSF is in direct contact with brain, it offers the best potential as a reliable source of biomarkers to be used in future diagnostic aids.

All of the animal experiments were conducted according to the Council of Europe and Finnish guidelines and approved by the State Provincial Office of Eastern Finland.

5. RESULTS

5.1 ASSOCIATION BETWEEN CSF Aβ42 AND NEUROPATHOLOGY IN MICE

The CSF Aβ42 levels increased significantly in APPswe/PS1 mice from age 5 months to 7 months ($p<0.001$). None of the mice showed any amyloid deposits in the histology at this stage. Between the age of 8 to 13 months, the CSF Aβ42 levels remained stable, and at the age of 13 months a few amyloid deposits could be detected in all mice. The APPswe/PS1dE9 mice had a few amyloid deposits already at the age of 6 months, but a severe amyloid pathology was observed only from the 9 months old mice. The CSF Aβ42 levels of 6 months old APPswe/PS1dE9 mice was comparable to the 13 months old APPswe/PS1 mice. The CSF Aβ42 levels decreased significantly during this time that the amyloid pathology advanced rapidly and the levels in 9 months old mice were significantly lower than the levels in 6 months old mice ($p<0.001$). Taken together, the CSF Aβ levels increased before the development of amyloid pathology and reached a plateau where the levels remained until the levels decreased when full blown amyloid pathology developed.

The CSF Aβ42 levels of 5 months old APPswe/PS1 mice correlated with the HC insoluble Aβ42 at the age of 7 months ($r=0.48$, $p=0.03$). Also the CSF Aβ42 levels of the 8 months old mice correlated with the HC insoluble Aβ42 of the 13 months old mice ($r=0.53$. $p<0.001$). The CSF levels that were measured from mice at the 13 months when the tissue sample was obtained showed weaker correlation ($r=0.33$, $p=0.04$). In the APPswe/PS1dE9 mice there was no correlation between the HC insoluble Aβ42 levels and the CSF Aβ42 levels at either time point.

5.2 CLINICAL CHARACTERISTICS OF HUMAN SUBJECTS

A total of 60 controls and 79 patients with MCI of whom 33 progressed to dementia were included in the study. Study **II** included 46 control subjects and 78 MCI patients. During the follow-up, 23 MCI patients developed AD. The age and gender distribution were similar in all groups. Study **III** included all of the patients from study **II**, except one control subject who was suffering from fibromyalgic pains and was cognitively healthy at the baseline but progressed to AD by the time of the last follow-up of study **III**. Study **III** included also one

additional MCI patient and the follow-up period was extended for many of the subjects. At the end of study **III**, the control group included 19 subjects with depression and 41 subjects with other neurological symptoms at the baseline. The MCI group consisted of 46 stable MCI patients and 33 who progressed to dementia. The age and gender distribution was similar between the groups. The follow-up period for the progressive patients ended at the time of diagnosis of dementia, and therefore the follow-up was shorter in the progressive MCI group. The presence of the ApoE 4 allele was more frequent in progressive MCI group than in the control or stable MCI groups. Table 3 presents the baseline demographic details of subjects in the different groups.

At the baseline of study **III,** the MCI patients were divided into three groups according to the type of the cognitive impairment. The largest group was the amnestic MCI patients with a total of 47 patients. Eight had isolated amnestic MCI, 39 had impairment in some other cognitive domain as well. Seventeen patients had executive MCI and 15 patients had impairment in some other cognitive domain, for example language or visuospatial skills. In all, 51% of the amnestic MCI patients, 41% of the executive MCI patients and 13% of the other MCI patients progressed to dementia during the follow-up. Twelve of the stable MCI patients had cognitive impairment due to vascular reasons, the etiology of 32 stable MCI patients causing the cognitive impairment remained undetermined. The most frequent diagnosis in the progressive MCI group was AD, but the progressive MCI group included also five patients with dementia of mixed etiology and one VaD patient.

Table 3. The clinical characteristics of studied controls (n=60) and MCI patients (n= 79) at the baseline.

	Controls			Stable MCI				Progressive MCI			
	All	**OND**	**Depression**	**All**	**Amnestic**	**Executive**	**Other**	**All**	**Amnestic**	**Executive**	**Other**
n	60	41	19	46	23	10	13	33	24	7	2
Age	68.3±8.1	69.4±6.8	66.0±10.2	69.5±8.1	70.0±7.7	68.4±7.3	69.2±9.9	71.7±6.7	71.1±7.1	74.3±5.8	69.0±4.2
Sex, men/women	25 / 36 42%/58%	17 / 24 41%/59%	8 / 11 42%/58%	20 / 26 44%/56%	11 / 12 48%/52%	7 / 3 70%/30%	2 / 11 15%/85%	13 / 20 39%/61%	10 / 14 42%/58%	2 / 5 29%/71%	1 / 1 50%/50%
MMSE	26.2±2.9 missing 9	26.7±2.5 missing 6	25.1±3.4 missing 3	24.1±2.5	23.3±3.1	24.3±1.8	24.2±1.7	23.9±2.7	23.8±2.9	23.9±1.9	25.5±3.5
Follow-up, years	3.94±2.22	3.85±2.13	4.13±2.45	4.57±3.09	3.87±2.63	5.90±4.23	4.77±2.71	3.52±1.95	3.50±2.09	3.00±1.41	5.50±0.71
ApoE4 +/-	21 / 39 35%/65%	13 / 28 32%/68%	8 / 11 42%/58%	15 / 30 33%/67% missing 1	7 / 15 32%/68% missing 1	3 / 7 30%/70%	5 / 8 38%/62%	26 / 7 79%/21%	20 / 4 83%/17%	4 / 3 57%/43%	2 / 0 100%/0%
Etiology				12 VCI 2 FD 32 NAS	6 VCI 1 FD 16 NAS	4 VCI 6 NAS	2 VCI 1 FD 10 NAS	27 AD 5 Mixed dementia 1 VaD	21 AD 3 Mixed dementia	5 AD 2 Mixed dementia	1 AD, atypical 1 VaD

Data presented as mean ± SD or number of subjects and percentage of all subjects in the group. MCI, mild cognitive impairment; OND, other neurological diseases; VCI, vascular cognitive impairment; FD, suspected frontal degeneration; NAS, non aliter specificatus; AD, Alzheimer's disease; VaD, vascular dementia.

5.3 CEREBROSPINAL FLUID BIOMARKER LEVELS AT BASELINE

In studies **II** and **III,** the CSF Aβ42, tau and phospho-tau levels were not statistically different between the controls and stable MCI patients. However, in progressive MCI patients, the CSF Aβ42 levels did decrease and tau and phospho-tau levels increased when compared either controls or stable MCI patients.

The CSF biomarker levels were not different between men and women. In study **III**, tau levels correlated with age in the control group (r=0.290, p=0.025), but none of the measured biomarkers correlated with age in the whole study group or any other diagnostic group. However, the ApoE genotype was found to have an effect on all of the studied biomarkers in the control group and on Aβ42 in the progressive MCI group.(Figure 2)

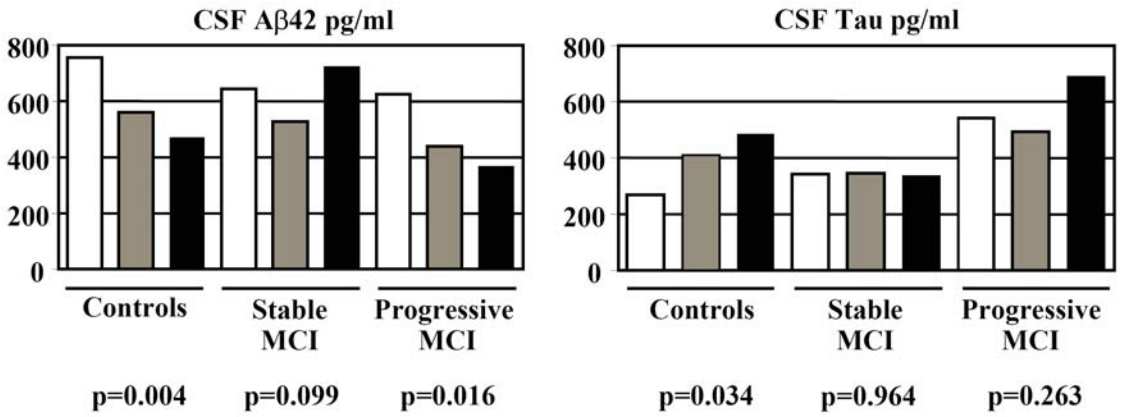

Figure 2. CSF levels of Aβ42 and tau in different diagnostic groups. White bars: subjects with no ApoE 4 alleles (n=76), grey bars: subjects with one ApoE 4 allele (n=44) and black bars: subjects with two ApoE 4 alleles (n=18).

5.4 CUT-OFF VALUES OF DIFFERENT BIOMARKERS FOR DEVELOPING AD

The cut-off values for different biomarkers were determined by using a ROC analysis. The analysis included all controls subjects and progressive MCI subjects from study **II**. Figure 3 shows an example of the result of Aβ42 ROC analysis in a visual form.

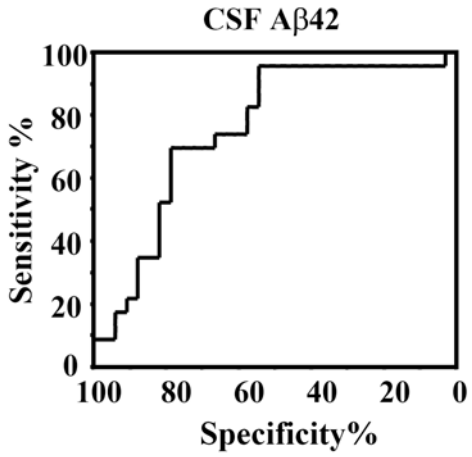

Figure 3. The ROC results for Aβ42. The analysis included 46 controls and 23 MCI patients who developed AD during the study.

The best cut-off value was the point on the curve that had the highest sensitivity and specificity, in the case of Aβ42 the value was 452 pg/ml, area under curve (AUC) 0.831 (0.726-0.935). The cut-off value for tau was 399 pg/ml, AUC 0.869 (0.786-0.951) and phospho-tau 70 pg/ml, AUC 0.839 (0.744-0.935). The test for Aβ42 was positive if the CSF Aβ42 level was below the cut-off value and for tau and phospho-tau if the CSF levels were above the cut-off value. These cut-off values were used to determine the sensitivity and specificity of these biomarkers for progressive MCI.(Table 4) Also the 5 mixed dementia subjects and the one VaD patient were included in the analysis.

5.5 PREDICTORS OF DEVELOPING DEMENTIA

The predictive value of different clinical measures was determined by logistic regression analysis on study **III**. The analysis included only MCI patients. The patients' age, sex or MMSE score at the baseline were not significant predictors for dementia. The patients carrying the ApoE 4 allele were about 7 times more likely to develop dementia during the study. The patients with two ApoE 4 alleles were also younger at the time of dementia diagnosis.

Table 4. The characteristics of different measures as a diagnostic test for developing dementia.

	Controls Specificity	Stable MCI Specificity	Progressive MCI Sensitivity	Overall Accuracy	Positive LR Controls Stable MCI	Negative LR Controls Stable MCI
Aβ42	90.0%	80.4%	57.6%	79.1%	5.76 (2.55 - 12.99)	0.47 (0.31 - 0.71)
	54 / 60	37 / 46	19 / 33	110 / 139	2.94 (1.53 - 5.67)	0.53 (0.35 - 0.81)
Tau	76.7%	63.0%	84.8%	74.1%	3.64 (2.25 - 5.88)	0.20 (0.09 - 0.45)
	46 / 60	29 / 46	28 / 33	103 / 139	2.30 (1.53 - 3.44)	0.24 (0.10 - 0.56)
Phospho-tau	66.7%	60.9%	84.8%	69.1%	2.55 (1.73 - 3.74)	0.23 (0.10 - 0.52)
	40 / 60	28 / 46	28 / 33	96 / 139	2.17 (1.47 - 3.20)	0.25 (0.11 - 0.58)
Aβ42 + tau	93.3%	89.1%	48.5%	82.0%	7.73 (2.83 - 21.07)	0.52 (0.36 - 0.74)
	56 / 60	41 / 46	17 / 33	114 / 139	4.74 (1.94 - 11.56)	0.54 (0.38 - 0.78)
Aβ42 + phospho-tau	93.3%	89.1%	48.5%	81.3%	7.27 (2.65 - 19.97)	0.55 (0.39 - 0.77)
	56 / 60	41 / 46	16 / 33	113 / 139	4.46 (1.81 - 10.96)	0.58 (0.41 - 0.82)
HC atrophy		61.5%	87.5%	71.4%	NA*	NA*
		8 / 13	7 / 8	15 / 21	2.28 (1.09 - 4.75)	0.20 (0.03 - 1.34)

*Data presented as percentage and correctly allocated subjects of all subjects in the group or as LR (95% CI). The table also includes these figures of MTL atrophy (at least one of the HC was atrophied) of the 21 patients from study **IV**. The first LR indicates the results when progressive MCI patients were compared to controls and the second LR indicates the results when the progressive MCI patients were compared to stable MCI patients. LR, likelihood ratio, MCI, mild cognitive impairment. *NA not applicable.*

The amnestic MCI and executive MCI patients were over 10 times more likely to develop dementia than patients with other types of cognitive impairment.(Figure 4) There was no significant difference in progression to dementia between the amnestic MCI patients and executive MCI patients.

All of the studied CSF biomarkers were significant predictors of dementia alone, but in the backward logistic regression CSF tau was the only biomarker that remained significant. In order to obtain more reliable results on the predictivity of the CSF biomarkers for development of dementia, the survival analysis of MCI patients with different numbers of abnormal biomarker values (Aβ42 or tau) was conducted by including all of the patients from

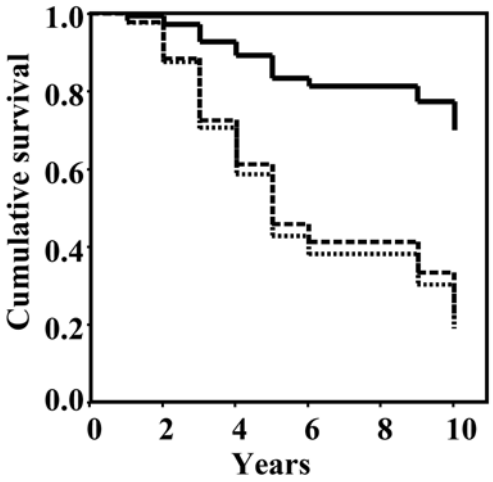

Figure 4. The survival curves of 79 MCI patients divided into groups according to the type of their cognitive impairment. The end point event was the development of dementia. The solid line represents the patients with other types of cognitive decline, the dashed line represents amnestic MCI patients and the dotted line represents executive MCI patients.

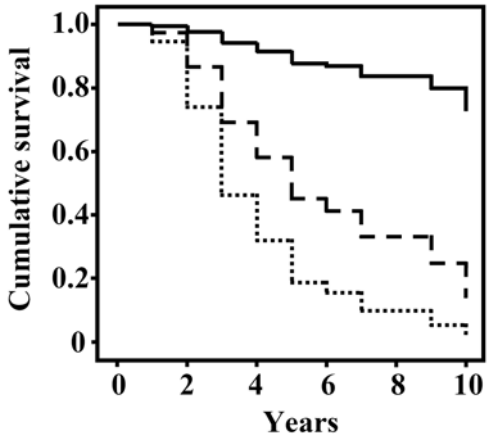

Figure 5. Survival curves of 80 MCI patients (79 from study **III** and one from study **IV**) divided into groups according to CSF Aβ42 and tau values. The end point event was the development of dementia. The solid line represents the patients with normal biomarker values, the dashed line represents patients with either abnormal Aβ42 of tau value and the dotted line represents patients with both abnormal Aβ42 and tau values.

study **III** and one additional patient from study **IV.** The follow-up period was also one to two years longer in eight stable MCI patients from study **II**. Figure 5 shows that MCI patients who have normal CSF biomarker values progress to dementia less frequently than those MCI patients who have either abnormal CSF levels of Aβ42 or tau. Almost all of the MCI patients who have both abnormal Aβ42 and tau develop dementia, over half of them during the first five years after the sample collection.

5.6 ASSOCIATION BETWEEN CSF BIOMARKERS AND MEDIAL TEMPORAL LOBE ATROPHY

In the whole study group, CSF tau levels correlated inversely with both right and left HC and volume of left EC. The results of phospho-tau were virtually identical. CSF Aβ42 correlated with the volume of left HC. When the groups were analysed separately, in the stable MCI patients tau correlated with volumes of both hippocampi and with left EC volume. The results of phospho-tau were otherwise similar, but the correlation with left EC did not reach statistical significance. In the progressive MCI patients, left HC volume correlated with CSF Aβ42 and phospho-tau. Word list delayed recall correlated positively with both right and left HC volumes and inversely with CSF tau and phospho-tau. In the subgroup analyses, there were no significant correlations in the stable MCI patients whereas there was a significant correlation between delayed recall and left hippocampal volume in the progressive MCI patients.

6. DISCUSSION

6.1 CHANGES IN CSF Aβ42, TAU AND PHOSPHO-TAU OCCUR EARLY IN THE COURSE OF THE DISEASE

Since the neuropathological changes of AD may start to develop as early as two decades before the onset of symptoms, we hypothesized that the AD associated changes in CSF biomarkers would be evident before the clinical diagnosis of AD and could be used to predict the development of AD in MCI patients. Based on the results of studies **II** and **III**, this hypothesis was proven to be true. CSF Aβ42 levels were decreased and CSF tau and phospho-tau levels were increased in those MCI patients who received a diagnosis of AD during a follow-up period of approximately four years. It should be emphasized that these subjects could not be clinically separated from the stable MCI patients even though they underwent a thorough clinical and neuropsychological examination. The results indicate that the changes in CSF biomarkers are related to the presence of AD associated neuropathology in patients with progressing cognitive impairment before dementia. However, the variability in CSF biomarker levels between individual subjects in each group was large and there was a significant overlap between the groups.

6.2 BIOLOGICAL VALIDITY OF CSF BIOMARKERS

One of the most important criteria for a biomarker is that it should detect the fundamental feature of neuropathology of AD (Consensus report of the Working Group on: "Molecular and Biochemical Markers of Alzheimer's Disease". The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group. 1998). Previous studies in transgenic mice have suggested that the on-going amyloid pathology is reflected in plasma and CSF (DeMattos et al. 2002, Kawarabayashi et al. 2001). The results from study **I** on mice confirmed these findings. CSF Aβ42 levels in mice started to decline at the time when the progression of amyloid pathology accelerated in brain. Although it is not possible to directly extrapolate the results from the mice to human disease, these results imply that the amyloid plaques may well interfere with the clearance of Aβ42 from brain to CSF.

Few human studies that have examined the association between CSF Aβ42, tau and phospho-tau and AD neuropathology. One study examined *post mortem* ventricular CSF Aβ42 levels and found that lower levels correlated with amyloid pathology in brain and cerebral vessels (Strozyk et al. 2003). Other studies have shown that CSF tau or phospho-tau231 correlate with NFT score or levels of hyperphosphorylated tau in neocortex (Buerger et al. 2006a, Tapiola et al. 1997). One study on *ante mortem* CSF found that increased tau levels were associated with AD and decreased Aβ42 levels were associated with DLB and prion disease (Clark et al. 2003). However, the association was not assessed between the CSF biomarkers and quantitative amount of amyloid or tau pathology. One recent study examined the diagnostic accuracy of CSF Aβ42, tau and phospho-tau in 100 neuropathologically confirmed AD patients (Engelborghs et al. 2007). The CSF biomarkers helped to distinguish seven out of nine of those AD patients whose clinical diagnosis had remained uncertain. Again, CSF Aβ42 levels were lower and tau and phospho-tau levels higher in the AD patients when compared to controls, but the association of the CSF biomarkers to the quantitative amount of pathology was not reported. Even though the current data is sparse, it seems that these biomarkers reflect AD associated neuropathological changes. Our own preliminary data from a large clinicopathological study supports this belief, the *ante mortem* biomarkers correlated with quantitative amyloid and NFT pathology in the neuropathological examination.

Our own longitudinal study showed that the CSF Aβ42 levels decreased during a three year period, possibly reflecting the aggravation of the amyloid pathology in the brain (Tapiola et al. 2000b). Other human data support also the association between the CSF Aβ42 and amyloid pathology. Molecular imaging of amyloid using amyloid binding compound PIB in living subjects has been shown to reflect amyloid accumulation in the brain (Kemppainen et al. 2006). The reduced CSF Aβ levels are associated with increased PIB binding in brain, which supports the association of a decline in CSF Aβ42 levels and extent of amyloid pathology (Fagan et al. 2006). Furthermore, in a study combining CSF biomarkers and PIB analysis, all of the subjects whose CSF Aβ42 level was below 457 pg/ml displayed also positive PIB binding and those whose levels were above that level showed negative PIB binding (Fagan et al. 2007). This value is virtually the same as the calculated cut-off value for Aβ42 in study **II**.

Thus the current data indicate that CSF biomarkers are strongly associated with the AD

associated neuropathology. However, the mechanisms involved in the development of CSF changes have remained unclear. Several mechanisms may influence CSF Aβ42 levels during the development of AD. The reduced levels may be due to the decreased synthesis, increased elimination or decreased clearance into CSF from the brain. Metabolism of Aβ42 is a very dynamic process. A recent study showed that Aβ42 is rapidly produced and cleared from the brain (Bateman et al. 2006). Therefore even very slight changes in the balance between the synthesis and elimination might lead to accumulation of amyloid in brain as plaques. Many studies, including our study **III,** have shown that the ApoE 4 allele has a dose dependent effect on CSF Aβ42 levels (Prince et al. 2004, Sunderland et al. 2004, Tapiola et al. 2000a). Furthermore, the CSF Aβ42 levels decrease faster over an average of a four year period in ApoE 4 positive subjects than in the ApoE 4 negative subjects (Huey et al. 2006). The association between the ApoE and neuropathology of AD is complex but the neuropathological studies have suggested that ApoE 4 can enhance Aβ aggregation (Gomez-Isla et al. 1996, Moir et al. 1999, Polvikoski et al. 1995) which in turn might lead to decreased CSF Aβ levels. Recent data also indicate that CSF Aβ42 levels show an hour-to-hour variation within individuals, with the trend for increasing levels starting from morning (Bateman et al. 2007). The changes seem large enough to have possible implications on diagnostic use and may require standardization of protocols with respect to time for collecting CSF samples.

6.3 CSF BIOMARKERS AS DIAGNOSTIC AIDS

Our results revealed that the combination of decreased CSF Aβ42 and increased tau or phospho-tau predicted relatively accurately the development of AD during a follow-up of a few years. Another recent study also reported that the combination of CSF Aβ42 and tau was a good predictor of developing AD (Hansson et al. 2006). The high positive likelihood ratio (LR) indicates that the abnormal result in the combination test confirms AD in mildly impaired patients with high accuracy. Only 7% of the controls and 11% of the stable MCI patients who had both abnormal Aβ42 and tau did not develop dementia. However, these subjects may be false positives because it is impossible to exclude AD associated neuropathological changes even in clinically healthy controls, much less in the stable MCI patients. Many studies have shown that apparently asymptomatic subjects may display brain pathology that fulfils the neuropathological criteria for AD (Bennett et al. 2006, Galvin et al.

2005, Neuropathology Group. Medical Research Council Cognitive Function and Aging Study 2001, Price and Morris 1999). The lack of a golden standard, the neuropathological diagnosis, is a problem in most biomarker studies performed on living subjects. This may also result in low accuracy in the differentiation between AD and other dementias since AD pathology is a common finding in subjects with VaD and DLB (Holmes et al. 1999, Londos et al. 2001, Neuropathology Group. Medical Research Council Cognitive Function and Aging Study 2001). It is possible that changes in CSF Aβ42 and tau reflect the presence of neuropathology of AD in subjects that receive another clinical diagnosis. One way to improve the accuracy of the clinical diagnosis is to use a long follow-up. The one control subject who entered the study as a healthy control but was later excluded from study due to development of AD emphasizes this problem in cross-sectional studies. Her measured biomarkers were clearly abnormal at the stage when her cognitive capability was normal, but after ten years follow-up she developed AD. Also others have reported decreased CSF Aβ42 levels in cognitively healthy subjects who later developed AD (Skoog et al. 2003). Although the follow-up period in our study, approximately four years, was longer than in most of the previous MCI studies, it would need to be even longer to reliably exclude all patients with developing AD. Thus it is possible that our stable MCI group also includes a few patients who will ultimately progress to dementia.

The present clinical criteria for AD date from 1993 and progress in both clinical and biomedical research has implications for revising the criteria. Currently, symptomatic medications for AD are widely used, but also disease modifying treatments are under development. Numerous compounds have entered preclinical studies and phase I and II clinical trials, and there are as many as twelve different compounds undergoing phase III trials for either slowing or attenuating the disease progression (Alzheimer Research Forum). Many of these compounds are drugs that are already in clinical use for other indications, for example statins and non-steroidal anti-inflammatory drugs (NSAIDs). Two of these test compounds may influence metabolism of Aβ. Tramiprosate (Alzhemed^{TM}) binds to Aβ and prevents the formation of amyloid fibrils in transgenic mice (Alzheimer Research Forum). R-flurbiprofen (Flurizan^{TM}) belongs to the NSAID group, but it is the only member of that class of compounds that has proven effect on Aβ production since it selectively modulates γ-secretase activity, decreasing the production of Aβ42 (Eriksen et al. 2003). Although the first Aβ vaccination trial was prematurately terminated due to side effects, less toxic Aβ

immunotherapeutic approaches are under development (Brendza and Holtzman 2006, Schenk 2002, Senior 2002). It is likely that any disease modifying treatment would be most helpful if it could be initiated at an early stage of the disease. However, although the current clinical criteria work relatively well in advanced AD, they are not suitable for providing an early diagnosis. The discussion on updating the criteria is already underway wit the key objective being to improve the early diagnosis of AD. One issue in the debate is the role of CSF biomarkers and other paraclinical tests in AD diagnostics. Although there are still many unresolved issues in understanding the characteristics of CSF Aβ42 and tau, a knowledge of their levels may be helpful for clinicians. However, due to their low sensitivity, CSF biomarkers will only serve as a supportive test for positive diagnosis, not as a crucial part of the standard diagnostic criteria.

6.4 ASSOCIATION BETWEEN CSF BIOMARKERS AND STRUCTURAL CHANGES IN MEDIAL TEMPORAL LOBE

MTL atrophy has also been intensively evaluated as a possible biomarker for AD and several studies have shown that the presence of MTL atrophy predicts progression of MCI and development of AD (Bouwman et al. 2006, Jack et al. 1999, Killiany et al. 2000). Study **IV** showed that CSF biomarkers, especially tau and phospho-tau were significantly correlated with MTL atrophy. The data in the literature is sparse and contradictory. One study reported a significant relationship between CSF phospho-tau231 and HC volume in 22 AD patients, whereas another study on 88 patients found no correlation between these parameters (Hampel et al. 2005, Schonknecht et al. 2003). A study with 18 MCI patients and controls reported an inverse correlation between CSF phospho-tau231 and HC volume, whereas a positive correlation in AD patients was found in another study (Hampel et al. 2005, de Leon et al. 2004). One recent study using a visual rating of MTL atrophy instead of volumetric analysis reported an inverse relationship between MTL atrophy and CSF Aβ42, but no correlation to CSF tau in 39 MCI patients (Schoonenboom et al. 2005). The data from study **IV** should be considered very cautiously due to the small sample size. However, it is intriguing, that CSF tau levels correlated with MTL atrophy. CSF tau has been suggested to represent a marker for axonal and neuronal damage (Blennow et al. 1995). It is possible that CSF tau reflects the rate of neuronal cell death and axonal damage predominantly in MTL structures, which would fit well to the model of progression of NFT pathology in AD (Braak and Braak 1991). Another possibility is that the correlation arises from the presence of AD per se since both markers

reflect the disease.

In conclusion, these studies support the hypothesis that CSF tau may reflect neurodegeneration in AD. However, the number of patients in the studies is small and thus it is not possible to examine the added value of each of these biomarkers in the diagnosis of AD.

6.5 CLINICAL IMPLICATIONS

In addition to having devastating effects on life of the individual patients and their families, AD also poses enormous socioeconomic burden the society. It is estimated that in 2005 there were over 29 million people who had dementia and worldwide societal costs were US$ 315 billion, over US$ 100 billion in European Union alone (Wimo et al. 2007). As the population ages, the number of patients and costs of care will increase even further. The undiagnosed AD patient is more expensive for society than the patient with a diagnosis and appropriate treatment (Hill et al. 2002). Therefore it is essential to detect the AD patients as early as possible out of the vast pool of patients who may express concern about cognitive impairment. Predicting the prognosis of MCI based on neuropsychological features is not possible without follow-up examinations to reveal a decline. Progression to AD and other dementias varies greatly between studies due to different definitions of MCI, differences in populations studied and length of the follow-up period. Therefore, the design of this study was practical, based on a "real life" situation. The majority of the subjects were recruited from the memory clinic and the inclusion criteria were intentionally relatively wide. The MCI was not defined according to strict criteria of amnestic MCI but also patients with declines in other cognitive domains were included. Nonetheless, a detailed assessment of several cognitive domains was performed, and the effect of the type of cognitive impairment on progression was also examined.

Many studies have shown that the most consistent predictor for AD and other dementias is memory performance during a long follow-up. AD patients exhibit problems in both visual and verbal memory as early as ten years before the onset of dementia (Elias et al. 2000, Kawas et al. 2003, Tierney et al. 2005). The decline in other cognitive domains including fluency, speed and attention becomes apparent closer to the diagnosis (Saxton et al. 2004, Tierney et al. 2005). Studies with a short follow-up period have shown inconsistent results. Some studies have found that the amnestic symptoms are the best indicators for predicting

dementia (Busse et al. 2003, DeCarli et al. 2004, Perri et al. 2007). On the contrary, the predictive value of amnestic symptoms has been poor in many studies and patients with isolated memory impairment have shown low rates of progression to dementia (Bozoki et al. 2001, Meyer et al. 2002a, Sacuiu et al. 2005). A significant number of patients even improve during the follow-up (Ganguli et al. 2004). Furthermore, individual amnestic MCI patients progress to dementia with very distinct patterns of cognitive decline. It seems that the rate of progression varies and cognitive domains are affected in a different order (Hodges et al. 2006).

The results of study **III** indicated that executive MCI was frequent and these patients progressed to dementia even faster than amnestic MCI patients. This is probably due to the fact that a large proportion of the MCI patients was derived from the clinic and therefore might have been closer to the dementia stage of AD at the baseline. The progression rate to dementia was 51% in the amnestic MCI patients and 41% in the executive MCI patients. However, it needs to be noted that the follow-up period of the stable amnestic MCI patients was two years shorter than that of the executive MCI patients. Therefore the difference in progression rates to dementia might be higher if the amnestic MCI patients were followed as long as the executive MCI patients. Executive dysfunction has emerged as possible predictor of dementia also in other studies. Executive dysfunction is a common comorbidity factor in amnestic MCI patients (Loewenstein et al. 2006, Nordlund et al. 2005) and it may occur on its own without amnestic symptoms, as in our study (Royall et al. 2004, Tabert et al. 2006). Amnestic MCI patients with concomitant executive dysfunction also convert to dementia at higher a rate than patients with isolated amnestic symptoms (DeCarli et al. 2004).

The rarity of isolated amnestic MCI suggests that in the clinical setting the patients who complain of cognitive problems other than memory disturbance should also be considered to be at high risk for dementia, particularly AD. The high frequency of executive MCI without amnestic symptoms in this study and the high progression rate to dementia of these patients strongly supports this belief. It has also been suggested that executive dysfunction influences the activities of daily living and thus the development of the full-blown dementia syndrome more than memory decline (Royall et al. 2005). Furthermore, almost half of the subjects who develop dementia within a three year period do not report memory problems in the clinic even when they are asked directly (Palmer et al. 2003). Therefore the amnestic MCI criteria exclude a high number of MCI patients who are at a high risk of developing AD and other

dementias. In summary, clinicians should not concentrate only on memory impairment in their evaluation of the predementia stage of AD. Nonetheless, it seems that even the most detailed single neuropsychological assessment cannot distinguish MCI patients who will develop dementia from those who will remain unchanged. Also, the causes of executive MCI may be more heterogeneous than those of amnestic MCI. Therefore one will need to resort to other diagnostic instruments such as CSF biomarkers are when trying to clarify the diagnosis and prognosis of the typical MCI patient.

6.6 LIMITATIONS OF THE STUDY

The study population of this study included subjects from both a population based cohort and from the memory clinic. Therefore the neuropsychological tests that were used for assessing different cognitive domains, especially memory, varied between different patients. However, the normative values for the different memory tests were available. The inclusion of subjects from the memory clinic may lead to a potential selection bias. Although all patients with cognitive complaints were invited to participate in the study, many declined and it is possible that those with more severe symptoms were more willing to enter the study. Also the inclusion of patients with depression may be problematic. Many behavioural symptoms, especially depression, may precede memory impairment and dementia years before the onset of symptoms. Therefore it is possible that the control group included a few patients who may eventually progress to MCI and dementia. The etiology of MCI was not defined in large proportion of stable MCI patients. This may be partly due to the fact that most patients underwent CT imaging instead of MRI and therefore mild atrophy or vascular pathology could not be detected. Thus, many of the stable MCI patients may have been suffering from VCI. It is also impossible to exclude mild depression or temporary use of some medication that could slightly impair cognitive function. Furthermore, when the study was initiated, the criteria for MCI included the cognitive impairment beyond age-adjusted normative values, not impairment over a period of time. Thus, some stable MCI patients may have had a low basic level of cognitive functions for all their lives but the values would low enough that they were included in the study. The follow-up period was longer than in most previous studies, but an even longer time would be needed to reliably detect all patients who will eventually develop AD. Also, the final diagnosis of AD was based on clinical criteria, and no neuropathological confirmation of the diagnosis was undertaken. Although the number of studied subjects was quite substantial, it is too small to accurately assess the added value of CSF biomarkers as

diagnostic tests for AD.

There are also some methodological limitations. Considering the recent data on hour-to-hour variability of CSF Aβ42 values within the same individual, (Bateman et al. 2007) one possible limitation of this study is that the time of the CSF sampling was not standardized. The CSF samples were collected mainly at midday or in the early afternoon, which is also the time that out-patient clinic CSF samples are taken in general. The standard polypropylene tubes were used to prevent the artefactually low Aβ42 level due to binding to the tubes. The samples were immediately aliquoted and frozen, and they were stored at -70°C until analyses. Some of the samples were frozen for up to a decade but no association between the storage time and CSF Aβ42, tau and phospho-tau was detected. The intra-assay variation was not assessed during the study. However, these assays are currently provided as a diagnostic service and run routinely in the same department where the study was conducted. The long term variability has remained good, median intra-assay CV% being 14.6% for Aβ42, 8.3% for tau and 6.7% for phospho-tau.

In the context of the studies on mice it needs to be taken into account that the physiology of the mouse is different from the human and the results of these studies cannot be extrapolated to humans directly. The transgenic mice are even further divergent from humans, because their normal physiology is disturbed by the inclusion of two genes with different mutations. The resulting changes in different studied parameters are most likely due to the influence of these inserted genes, but it is also possible that the transgene construct is inserted in a way that it disturbs the function of some endogenous gene. However, both of the mouse lines that were used in this study have been well characterized, and the development of amyloid pathology as a result of the mutated APP and PS1 genes has been described in numerous other transgenic mouse lines as well (Borchelt et al. 1997, Jankowsky et al. 2004, Spires and Hyman 2005). The development of amyloid pathology in mice is different from the neuropathological process in human brains. In transgenic mice, the main reason for Aβ accumulation is the increased production of Aβ, whereas in human brain, especially in sporadic AD, the accumulation is a consequence of several factors, including decreased degradation and reduced clearance of Aβ. Furthermore, the mice do not exhibit abundant vascular amyloid pathology or age related changes in brain. It could be speculated that these common changes including atherosclerosis, are also important contributors to the

neurodegenerative process in humans but are not present in the mouse models. The amyloid pathology also develops very rapidly in the mice, during a 6-month time frame, whereas in humans the pathology appears over a period as long as two decades. Another limitation of the mice that were used in this study is the fact that they do not develop tau pathology. Therefore the study examined only the association between the CSF Aβ42 and amyloid pathology in these mice. However, the results of study **I** supported the data on human studies; the CSF Aβ42 levels decreased simultaneously with the increasing amyloid pathology.

7. CONCLUSIONS

The aim of the present study was to investigate whether the CSF Aβ42, tau and phospho-tau can be used as diagnostic aid in diagnosis of early AD. The secondary aim was to examine the relationship between the CSF biomarkers and neuropathological processes associated with AD. The following conclusions can be drawn:

1. AD associated changes in CSF biomarkers, i.e. decrease in Aβ42 and increase in tau and phospho-tau occur during a predementia stage of AD.

2. CSF Aβ42, tau and phospho-tau values are associated with MTL atrophy. CSF tau levels may reflect the neuronal damage occurring in MTL.

3. ApoE 4 allele has a dose dependent effect on CSF biomarkers such that the Aβ42 levels decrease and tau and phospho-tau levels increase with elevating numbers of ApoE 4 alleles.

4. A large proportion of MCI patients do not have memory impairment. Executive dysfunction without amnestic symptoms is common, and many of these patients progress to AD. In the clinical context, patients with cognitive impairment without memory complaint should be also considered to be at a high risk for developing AD.

5. Several years before the any clinical diagnosis can possibly be made, the combination of decreased CSF Aβ42 and increased tau or phospho-tau can differentiate with high specificity those MCI patients who will eventually develop AD from those who remain stable. The positive test result can be used as a confirmation of a clinical diagnosis of possible early AD. However, the sensitivity of the test is low and a negative result cannot exclude the presence of AD.

REFERENCES

Consensus report of the Working Group on: "Molecular and Biochemical Markers of Alzheimer's Disease". The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group. Neurobiol Aging 1998;19:109-116.

Aggarwal NT, Wilson RS, Beck TL, Bienias JL, Bennett DA. Motor dysfunction in mild cognitive impairment and the risk of incident Alzheimer disease. Arch Neurol 2006;63:1763-1769.

Alonso A, Zaidi T, Novak M, Grundke-Iqbal I, Iqbal K. Hyperphosphorylation induces self-assembly of tau into tangles of paired helical filaments/straight filaments. Proc Natl Acad Sci USA 2001;98:6923-6928.

Alvarez G, Munoz-Montano JR, Satrustegui J, Avila J, Bogonez E, Diaz-Nido J. Lithium protects cultured neurons against beta-amyloid-induced neurodegeneration. FEBS Lett 1999;453:260-264.

Alzheimer research forum. Drugs in clinical trials [Homepage of Alzheimer Research Forum], [Online]. Available: http://www.alzforum.org/drg/drc/default.asp [April 30th, 2007].

American psychiatric association. Diagnostic and statistical manual of mental disorders. 1994 4th edn. Washington: APA.

Ancelin ML, Artero S, Portet F, Dupuy AM, Touchon J, Ritchie K. Non-degenerative mild cognitive impairment in elderly people and use of anticholinergic drugs: longitudinal cohort study. BMJ 2006;332:455-459.

Anchisi D, Borroni B, Franceschi M, Kerrouche N, Kalbe E, Beuthien-Beumann B, Cappa S, Lenz O, Ludecke S, Marcone A, Mielke R, Ortelli P, Padovani A, Pelati O, Pupi A, Scarpini E, Weisenbach S, Herholz K, Salmon E, Holthoff V, Sorbi S, Fazio F, Perani D. Heterogeneity of brain glucose metabolism in mild cognitive impairment and clinical progression to Alzheimer disease. Arch Neurol 2005;62:1728-1733.

Andreasen N, Minthon L, Vanmechelen E, Vanderstichele H, Davidsson P, Winblad B, Blennow K. Cerebrospinal fluid tau and Abeta42 as predictors of development of Alzheimer's disease in patients with mild cognitive impairment. Neurosci Lett 1999;273:5-8.

Andreasen N, Vanmechelen E, Van de Voorde A, Davidsson P, Hesse C, Tarvonen S, Raiha I, Sourander L, Winblad B, Blennow K. Cerebrospinal fluid tau protein as a biochemical marker for Alzheimer's disease: a community based follow up study. J Neurol Neurosurg Psychiatry 1998;64:298-305.

Arai H, Ishiguro K, Ohno H, Moriyama M, Itoh N, Okamura N, Matsui T, Morikawa Y, Horikawa E, Kohno H, Sasaki H, Imahori K. CSF phosphorylated tau protein and mild cognitive impairment: a prospective study. Exp Neurol 2000;166:201-203.

Arai H, Morikawa Y, Higuchi M, Matsui T, Clark CM, Miura M, Machida N, Lee VM, Trojanowski JQ, Sasaki H. Cerebrospinal fluid tau levels in neurodegenerative diseases with distinct tau-related pathology. Biochem Biophys Res Commun 1997;236:262-264.

Archer HA, Edison P, Brooks DJ, Barnes J, Frost C, Yeatman T, Fox NC, Rossor MN. Amyloid load and cerebral atrophy in Alzheimer's disease: an 11C-PIB positron emission tomography study. Ann Neurol 2006;60:145-147.

Arnaiz E, Almkvist O, Ivnik RJ, Tangalos EG, Wahlund LO, Winblad B, Petersen RC. Mild cognitive impairment: a cross-national comparison. J Neurol Neurosurg Psychiatry 2004;75:1275-1280.

Assini A, Cammarata S, Vitali A, Colucci M, Giliberto L, Borghi R, Inglese ML, Volpe S, Ratto S, Dagna-Bricarelli F, Baldo C, Argusti A, Odetti P, Piccini A, Tabaton M. Plasma levels of amyloid beta-protein 42 are increased in women with mild cognitive impairment. Neurology 2004;63:828-831.

Avila J. Tau phosphorylation and aggregation in Alzheimer's disease pathology. FEBS Lett 2006;580:2922-2927.

Bacskai BJ, Frosch MP, Freeman SH, Raymond SB, Augustinack JC, Johnson KA, Irizarry MC, Klunk WE, Mathis CA, Dekosky ST, Greenberg SM, Hyman BT, Growdon JH. Molecular imaging with Pittsburgh Compound B confirmed at autopsy: a case report. Arch Neurol 2007;64:431-434.

Baki L, Shioi J, Wen P, Shao Z, Schwarzman A, Gama-Sosa M, Neve R, Robakis NK. PS1 activates PI3K thus inhibiting GSK-3 activity and tau overphosphorylation: effects of FAD mutations. EMBO J 2004;23:2586-2596.

Bateman RJ, Munsell LY, Morris JC, Swarm R, Yarasheski KE, Holtzman DM. Human amyloid-beta synthesis and clearance rates as measured in cerebrospinal fluid in vivo. Nat Med 2006;12:856-861.

Bateman RJ, Wen G, Morris JC, Holtzman DM. Fluctuations of CSF amyloid-beta levels: implications for a diagnostic and therapeutic biomarker. Neurology 2007;68:666-669.

Bennett DA, Schneider JA, Arvanitakis Z, Kelly JF, Aggarwal NT, Shah RC, Wilson RS. Neuropathology of older persons without cognitive impairment from two community-based studies. Neurology 2006;66:1837-1844.

Bennett DA, Schneider JA, Bienias JL, Evans DA, Wilson RS. Mild cognitive impairment is related to Alzheimer disease pathology and cerebral infarctions. Neurology 2005;64:834-841.

Berg L, McKeel DW,Jr, Miller JP, Storandt M, Rubin EH, Morris JC, Baty J, Coats M, Norton J, Goate AM, Price JL, Gearing M, Mirra SS, Saunders AM. Clinicopathologic studies in cognitively healthy aging and Alzheimer's disease: relation of histologic markers to dementia severity, age, sex, and apolipoprotein E genotype. Arch Neurol 1998;55:326-335.

Blennow K. Cerebrospinal fluid protein biomarkers for Alzheimer's disease. NeuroRx 2004;1:213-225.

Blennow K and Hampel H. CSF markers for incipient Alzheimer's disease. Lancet Neurol

2003;2:605-613.

Blennow K, Wallin A, Agren H, Spenger C, Siegfried J, Vanmechelen E. Tau protein in cerebrospinal fluid: a biochemical marker for axonal degeneration in Alzheimer disease? Mol Chem Neuropathol 1995;26:231-245.

Bobinski M, de Leon MJ, Wegiel J, Desanti S, Convit A, Saint Louis LA, Rusinek H, Wisniewski HM. The histological validation of post mortem magnetic resonance imaging-determined hippocampal volume in Alzheimer's disease. Neuroscience 2000;95:721-725.

Bonelli RM, Aschoff A, Niederwieser G, Heuberger C, Jirikowski G. Cerebrospinal fluid tissue transglutaminase as a biochemical marker for Alzheimer's disease. Neurobiol Dis 2002;11:106-110.

Borchelt DR, Ratovitski T, van Lare J, Lee MK, Gonzales V, Jenkins NA, Copeland NG, Price DL, Sisodia SS. Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins. Neuron 1997;19:939-945.

Borkowski JG, Benton AL, Spreen O. Word fluency and brain damage. Neuropsychologia 1967;5:135-140.

Bouwman FH, Schoonenboom SN, van der Flier WM, van Elk EJ, Kok A, Barkhof F, Blankenstein MA, Scheltens P. CSF biomarkers and medial temporal lobe atrophy predict dementia in mild cognitive impairment. Neurobiol Aging 2006;28:1070-1074.

Bowler JV. Vascular cognitive impairment. J Neurol Neurosurg Psychiatry 2005;76 Suppl 5:35-44.

Boyle PA, Wilson RS, Aggarwal NT, Arvanitakis Z, Kelly J, Bienias JL, Bennett DA. Parkinsonian signs in subjects with mild cognitive impairment. Neurology 2005;65:1901-1906.

Bozoki A, Giordani B, Heidebrink JL, Berent S, Foster NL. Mild cognitive impairments predict dementia in nondemented elderly patients with memory loss. Arch Neurol 2001;58:411-416.

Braak H and Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol (Berl) 1991;82:239-259.

Brendza RP and Holtzman DM. Amyloid-beta immunotherapies in mice and men. Alzheimer Dis Assoc Disord 2006;20:118-123.

Buerger K, Ewers M, Pirttila T, Zinkowski R, Alafuzoff I, Teipel SJ, DeBernardis J, Kerkman D, McCulloch C, Soininen H, Hampel H. CSF phosphorylated tau protein correlates with neocortical neurofibrillary pathology in Alzheimer's disease. Brain 2006a;129:3035-3041.

Buerger K, Otto M, Teipel SJ, Zinkowski R, Blennow K, DeBernardis J, Kerkman D, Schroder J, Schonknecht P, Cepek L, McCulloch C, Moller HJ, Wiltfang J, Kretzschmar H, Hampel H. Dissociation between CSF total tau and tau protein phosphorylated at threonine 231 in Creutzfeldt-Jakob disease. Neurobiol Aging 2006b;27:10-15.

Buerger K, Teipel SJ, Zinkowski R, Blennow K, Arai H, Engel R, Hofmann-Kiefer K,

McCulloch C, Ptok U, Heun R, Andreasen N, DeBernardis J, Kerkman D, Moeller H, Davies P, Hampel H. CSF tau protein phosphorylated at threonine 231 correlates with cognitive decline in MCI subjects. Neurology 2002a;59:627-629.

Buerger K, Zinkowski R, Teipel SJ, Tapiola T, Arai H, Blennow K, Andreasen N, Hofmann-Kiefer K, DeBernardis J, Kerkman D, McCulloch C, Kohnken R, Padberg F, Pirttila T, Schapiro MB, Rapoport SI, Moller HJ, Davies P, Hampel H. Differential diagnosis of Alzheimer disease with cerebrospinal fluid levels of tau protein phosphorylated at threonine 231. Arch Neurol 2002b;59:1267-1272.

Busse A, Bischkopf J, Riedel-Heller SG, Angermeyer MC. Subclassifications for mild cognitive impairment: prevalence and predictive validity. Psychol Med 2003;33:1029-1038.

Butters N, Granholm E, Salmon DP, Grant I, Wolfe J. Episodic and semantic memory: a comparison of amnesic and demented patients. J Clin Exp Neuropsychol 1987;9:479-497.

Clark CM, Xie S, Chittams J, Ewbank D, Peskind E, Galasko D, Morris JC, McKeel DW,Jr, Farlow M, Weitlauf SL, Quinn J, Kaye J, Knopman D, Arai H, Doody RS, DeCarli C, Leight S, Lee VM, Trojanowski JQ. Cerebrospinal fluid tau and beta-amyloid: how well do these biomarkers reflect autopsy-confirmed dementia diagnoses? Arch Neurol 2003;60:1696-1702.

de Leon MJ, DeSanti S, Zinkowski R, Mehta PD, Pratico D, Segal S, Clark C, Kerkman D, DeBernardis J, Li J, Lair L, Reisberg B, Tsui W, Rusinek H. MRI and CSF studies in the early diagnosis of Alzheimer's disease. J Intern Med 2004;256:205-223.

de Mendonca A, Ribeiro F, Guerreiro M, Garcia C. Frontotemporal mild cognitive impairment. J Alzheimers Dis 2004;6:1-9.

DeCarli C, Mungas D, Harvey D, Reed B, Weiner M, Chui H, Jagust W. Memory impairment, but not cerebrovascular disease, predicts progression of MCI to dementia. Neurology 2004;63:220-227.

DeMattos RB, Bales KR, Parsadanian M, O'Dell MA, Foss EM, Paul SM, Holtzman DM. Plaque-associated disruption of CSF and plasma amyloid-beta (Abeta) equilibrium in a mouse model of Alzheimer's disease. J Neurochem 2002;81:229-236.

Dermaut B, Kumar-Singh S, Engelborghs S, Theuns J, Rademakers R, Saerens J, Pickut BA, Peeters K, van den Broeck M, Vennekens K, Claes S, Cruts M, Cras P, Martin JJ, Van Broeckhoven C, De Deyn PP. A novel presenilin 1 mutation associated with Pick's disease but not beta-amyloid plaques. Ann Neurol 2004;55:617-626.

Devanand DP, Pradhaban G, Liu X, Khandji A, De Santi S, Segal S, Rusinek H, Pelton GH, Honig LS, Mayeux R, Stern Y, Tabert MH, de Leon MJ. Hippocampal and entorhinal atrophy in mild cognitive impairment: prediction of Alzheimer disease. Neurology 2007;68:828-836.

Di Luca M, Pastorino L, Bianchetti A, Perez J, Vignolo LA, Lenzi GL, Trabucchi M, Cattabeni F, Padovani A. Differential level of platelet amyloid beta precursor protein isoforms: an early marker for Alzheimer disease. Arch Neurol 1998;55:1195-1200.

Driscoll I, Resnick SM, Troncoso JC, An Y, O'brien R, Zonderman AB. Impact of Alzheimer's pathology on cognitive trajectories in nondemented elderly. Ann Neurol 2006;60:688-695.

Ebneth A, Godemann R, Stamer K, Illenberger S, Trinczek B, Mandelkow E. Overexpression of tau protein inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum: implications for Alzheimer's disease. J Cell Biol 1998;143:777-794.

Edison P, Archer HA, Hinz R, Hammers A, Pavese N, Tai YF, Hotton G, Cutler D, Fox N, Kennedy A, Rossor M, Brooks DJ. Amyloid, hypometabolism, and cognition in Alzheimer disease: an [11C]PIB and [18F]FDG PET study. Neurology 2007;68:501-508.

Elias MF, Beiser A, Wolf PA, Au R, White RF, D'Agostino RB. The preclinical phase of alzheimer disease: A 22-year prospective study of the Framingham Cohort. Arch Neurol 2000;57:808-813.

Engelborghs S, De Vreese K, Van de Casteele T, Vanderstichele H, Van Everbroeck B, Cras P, Martin JJ, Vanmechelen E, De Deyn PP. Diagnostic performance of a CSF-biomarker panel in autopsy-confirmed dementia. Neurobiol Aging 2007 doi.org/10.1016/j.neurobiolaging.2007.02.016.

Eriksen JL, Sagi SA, Smith TE, Weggen S, Das P, McLendon DC, Ozols VV, Jessing KW, Zavitz KH, Koo EH, Golde TE. NSAIDs and enantiomers of flurbiprofen target gamma-secretase and lower Abeta 42 in vivo. J Clin Invest 2003;112:440-449.

Fagan AM, Mintun MA, Mach RH, Lee SY, Dence CS, Shah AR, LaRossa GN, Spinner ML, Klunk WE, Mathis CA, DeKosky ST, Morris JC, Holtzman DM. Inverse relation between in vivo amyloid imaging load and cerebrospinal fluid Abeta42 in humans. Ann Neurol 2006;59:512-519.

Fagan AM, Roe CM, Xiong C, Mintun MA, Morris JC, Holtzman DM. Cerebrospinal fluid tau/beta-amyloid(42) ratio as a prediction of cognitive decline in nondemented older adults. Arch Neurol 2007;64:343-349.

Farias ST, Mungas D, Reed BR, Harvey D, Cahn-Weiner D, Decarli C. MCI is associated with deficits in everyday functioning. Alzheimer Dis Assoc Disord 2006;20:217-223.

Fath T, Eidenmuller J, Brandt R. Tau-mediated cytotoxicity in a pseudohyperphosphorylation model of Alzheimer's disease. J Neurosci 2002;22:9733-9741.

Feldman H, Scheltens P, Scarpini E, Hermann N, Mesenbrink P, Mancione L, Tekin S, Lane R, Ferris S. Behavioral symptoms in mild cognitive impairment. Neurology 2004;62:1199-1201.

Fischer P, Jungwirth S, Zehetmayer S, Weissgram S, Hoenigschnabl S, Gelpi E, Krampla W, Tragl KH. Conversion from subtypes of mild cognitive impairment to Alzheimer dementia. Neurology 2007;68:288-291.

Fisk JD, Merry HR, Rockwood K. Variations in case definition affect prevalence but not outcomes of mild cognitive impairment. Neurology 2003;61:1179-1184.

Flicker C, Ferris SH, Reisberg B. Mild cognitive impairment in the elderly: predictors of dementia. Neurology 1991;41:1006-1009.

Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189-198.

Fukumoto H, Tennis M, Locascio JJ, Hyman BT, Growdon JH, Irizarry MC. Age but not diagnosis is the main predictor of plasma amyloid beta-protein levels. Arch Neurol 2003;60:958-964.

Galvin JE, Powlishta KK, Wilkins K, McKeel DW,Jr, Xiong C, Grant E, Storandt M, Morris JC. Predictors of preclinical Alzheimer disease and dementia: a clinicopathologic study. Arch Neurol 2005;62:758-765.

Ganguli M, Dodge HH, Shen C, DeKosky ST. Mild cognitive impairment, amnestic type: an epidemiologic study. Neurology 2004;63:115-121.

Ganguli M, Du Y, Dodge HH, Ratcliff GG, Chang CC. Depressive symptoms and cognitive decline in late life: a prospective epidemiological study. Arch Gen Psychiatry 2006;63:153-160.

Geslani DM, Tierney MC, Herrmann N, Szalai JP. Mild cognitive impairment: an operational definition and its conversion rate to Alzheimer's disease. Dement Geriatr Cogn Disord 2005;19:383-389.

Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, Fidani L, Giuffra L, Haynes A, Irving N, James L. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 1991;349:704-706.

Goedert M, Spillantini MG, Potier MC, Ulrich J, Crowther RA. Cloning and sequencing of the cDNA encoding an isoform of microtubule-associated protein tau containing four tandem repeats: differential expression of tau protein mRNAs in human brain. EMBO J 1989;8:393-399.

Goldman WP and Morris JC. Evidence that age-associated memory impairment is not a normal variant of aging. Alzheimer Dis Assoc Disord 2001;15:72-79.

Gomez-Isla T, West HL, Rebeck GW, Harr SD, Growdon JH, Locascio JJ, Perls TT, Lipsitz LA, Hyman BT. Clinical and pathological correlates of apolipoprotein E epsilon 4 in Alzheimer's disease. Ann Neurol 1996;39:62-70.

Gong CX, Liu F, Grundke-Iqbal I, Iqbal K. Post-translational modifications of tau protein in Alzheimer's disease. J Neural Transm 2005;112:813-838.

Gotz J, Chen F, van Dorpe J, Nitsch RM. Formation of neurofibrillary tangles in P301L tau transgenic mice induced by Aβ fibrils. Science 2001;293:1491-1495.

Graham JE, Rockwood K, Beattie BL, Eastwood R, Gauthier S, Tuokko H, McDowell I. Prevalence and severity of cognitive impairment with and without dementia in an elderly population. Lancet 1997;349:1793-1796.

Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI. Abnormal phosphorylation of the microtubule-associated protein tau in Alzheimer cytoskeletal pathology. Proc Natl Acad Sci USA 1986;83:4913-4917.

Guillozet AL, Weintraub S, Mash DC, Mesulam MM. Neurofibrillary tangles, amyloid, and memory in aging and mild cognitive impairment. Arch Neurol 2003;60:729-736.

Gustafson DR, Skoog I, Rosengren L, Zetterberg H, Blennow K. Cerebrospinal fluid A(beta)42 concentration may predict cognitive decline in elderly women. J Neurol Neurosurg Psychiatry 2006;78:461-464.

Haass C. Take five--BACE and the gamma-secretase quartet conduct Alzheimer's amyloid beta-peptide generation. EMBO J 2004;23:483-488.

Haass C, Schlossmacher MG, Hung AY, Vigo-Pelfrey C, Mellon A, Ostaszewski BL, Lieberburg I, Koo EH, Schenk D, Teplow DB. Amyloid beta-peptide is produced by cultured cells during normal metabolism. Nature 1992;359:322-325.

Hampel H, Buerger K, Zinkowski R, Teipel SJ, Goernitz A, Andreasen N, Sjoegren M, DeBernardis J, Kerkman D, Ishiguro K, Ohno H, Vanmechelen E, Vanderstichele H, McCulloch C, Moller HJ, Davies P, Blennow K. Measurement of phosphorylated tau epitopes in the differential diagnosis of Alzheimer disease: a comparative cerebrospinal fluid study. Arch Gen Psychiatry 2004;61:95-102.

Hampel H, Burger K, Pruessner JC, Zinkowski R, DeBernardis J, Kerkman D, Leinsinger G, Evans AC, Davies P, Moller HJ, Teipel SJ. Correlation of cerebrospinal fluid levels of tau protein phosphorylated at threonine 231 with rates of hippocampal atrophy in Alzheimer disease. Arch Neurol 2005;62:770-773.

Hampel H, Teipel SJ, Fuchsberger T, Andreasen N, Wiltfang J, Otto M, Shen Y, Dodel R, Du Y, Farlow M, Moller HJ, Blennow K, Buerger K. Value of CSF beta-amyloid1-42 and tau as predictors of Alzheimer's disease in patients with mild cognitive impairment. Mol Psychiatry 2004;9:705-710.

Hanninen T, Hallikainen M, Tuomainen S, Vanhanen M, Soininen H. Prevalence of mild cognitive impairment: a population-based study in elderly subjects. Acta Neurol Scand 2002;106:148-154.

Hanninen T, Koivisto K, Reinikainen KJ, Helkala EL, Soininen H, Mykkanen L, Laakso M, Riekkinen PJ. Prevalence of ageing-associated cognitive decline in an elderly population. Age Ageing 1996;25:201-205.

Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. Lancet Neurol 2006;5:228-234.

Hardy J and Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 2002;297:353-356.

Herholz K, Salmon E, Perani D, Baron JC, Holthoff V, Frolich L, Schonknecht P, Ito K, Mielke R, Kalbe E, Zundorf G, Delbeuck X, Pelati O, Anchisi D, Fazio F, Kerrouche N, Desgranges B, Eustache F, Beuthien-Baumann B, Menzel C, Schroder J, Kato T, Arahata Y, Henze M, Heiss WD. Discrimination between Alzheimer dementia and controls by automated analysis of multicenter FDG PET. Neuroimage 2002;17:302-316.

Hesse C, Rosengren L, Andreasen N, Davidsson P, Vanderstichele H, Vanmechelen E, Blennow K. Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. Neurosci Lett 2001;297:187-190.

Hill JW, Futterman R, Duttagupta S, Mastey V, Lloyd JR, Fillit H. Alzheimer's disease and related dementias increase costs of comorbidities in managed Medicare. Neurology 2002;58:62-70.

Hodges JR, Erzinclioglu S, Patterson K. Evolution of cognitive deficits and conversion to dementia in patients with mild cognitive impairment: a very-long-term follow-up study. Dement Geriatr Cogn Disord 2006;21:380-391.

Hogh P, Madsen Sjo N, Gade A, Waldemar G. Temporal lobe hypoperfusion in isolated amnesia with slow onset: a single photon emission computer tomography study. Dement Geriatr Cogn Disord 2004;18:15-23.

Holmes C, Cairns N, Lantos P, Mann A. Validity of current clinical criteria for Alzheimer's disease, vascular dementia and dementia with Lewy bodies. Br J Psychiatry 1999;174:45-50.

Hsiung GY, Sadovnick AD, Feldman H. Apolipoprotein E epsilon4 genotype as a risk factor for cognitive decline and dementia: data from the Canadian Study of Health and Aging. CMAJ 2004;171:863-867.

Huey ED, Mirza N, Putnam KT, Soares H, Csako G, Levy JA, Copenhaver B, Cohen RM, Sunderland T. Stability of CSF beta-amyloid(1-42) and tau levels by APOE genotype in Alzheimer patients. Dement Geriatr Cogn Disord 2006;22:48-53.

Hulstaert F, Blennow K, Ivanoiu A, Schoonderwaldt HC, Riemenschneider M, De Deyn PP, Bancher C, Cras P, Wiltfang J, Mehta PD, Iqbal K, Pottel H, Vanmechelen E, Vanderstichele H. Improved discrimination of AD patients using beta-amyloid(1-42) and tau levels in CSF. Neurology 1999;52:1555-1562.

Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, Pickering-Brown S, Chakraverty S, Isaacs A, Grover A, Hackett J, Adamson J, Lincoln S, Dickson D, Davies P, Petersen RC, Stevens M, de Graaff E, Wauters E, van Baren J, Hillebrand M, Joosse M, Kwon JM, Nowotny P, Che LK, Norton J, Morris JC, Reed LA, Trojanowski J, Basun H, Lannfelt L, Neystat M, Fahn S, Dark F, Tannenberg T, Dodd PR, Hayward N, Kwok JB, Schofield PR, Andreadis A, Snowden J, Craufurd D, Neary D, Owen F, Oostra BA, Hardy J, Goate A, van Swieten J, Mann D, Lynch T, Heutink P. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. Nature 1998;393:702-705.

Hwang TJ, Masterman DL, Ortiz F, Fairbanks LA, Cummings JL. Mild cognitive impairment is associated with characteristic neuropsychiatric symptoms. Alzheimer Dis Assoc Disord 2004;18:17-21.

Irizarry MC. Biomarkers of Alzheimer disease in plasma. NeuroRx 2004;1:226-234.

Iwatsubo T, Odaka A, Suzuki N, Mizusawa H, Nukina N, Ihara Y. Visualization of A beta 42(43) and A beta 40 in senile plaques with end-specific A beta monoclonals: evidence that an initially deposited species is A beta 42(43). Neuron 1994;13:45-53.

Jack CR,Jr, Dickson DW, Parisi JE, Xu YC, Cha RH, O'Brien PC, Edland SD, Smith GE, Boeve BF, Tangalos EG, Kokmen E, Petersen RC. Antemortem MRI findings correlate with hippocampal neuropathology in typical aging and dementia. Neurology 2002;58:750-757.

Jack CR,Jr, Petersen RC, Xu YC, O'Brien PC, Smith GE, Ivnik RJ, Boeve BF, Waring SC,

Tangalos EG, Kokmen E. Prediction of AD with MRI-based hippocampal volume in mild cognitive impairment. Neurology 1999;52:1397-1403.

Jankowsky JL, Fadale DJ, Anderson J, Xu GM, Gonzales V, Jenkins NA, Copeland NG, Lee MK, Younkin LH, Wagner SL, Younkin SG, Borchelt DR. Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide in vivo: evidence for augmentation of a 42-specific gamma secretase. Hum Mol Genet 2004;13:159-170.

Jensen M, Basun H, Lannfelt L. Increased cerebrospinal fluid tau in patients with Alzheimer's disease. Neurosci Lett 1995;186:189-191.

Jensen M, Schroder J, Blomberg M, Engvall B, Pantel J, Ida N, Basun H, Wahlund LO, Werle E, Jauss M, Beyreuther K, Lannfelt L, Hartmann T. Cerebrospinal fluid A beta42 is increased early in sporadic Alzheimer's disease and declines with disease progression. Ann Neurol 1999;45:504-511.

Jia JO, Meng R, Sun YX, Sun WJ, Ji WJ, Jia LF. Cerebrospinal fluid tau, Aβ1-42 and inflammatory cytokines in patients with Alzheimer's disease and vascular dementia. Neurosci Lett 2005;383:12-15.

Jicha GA, Parisi JE, Dickson DW, Johnson K, Cha R, Ivnik RJ, Tangalos EG, Boeve BF, Knopman DS, Braak H, Petersen RC. Neuropathologic outcome of mild cognitive impairment following progression to clinical dementia. Arch Neurol 2006;63:674-681.

Jobst KA, Barnetson LP, Shepstone BJ. Accurate prediction of histologically confirmed Alzheimer's disease and the differential diagnosis of dementia: the use of NINCDS-ADRDA and DSM-III-R criteria, SPECT, X-ray CT, and Apo E4 in medial temporal lobe dementias. Oxford Project to Investigate Memory and Aging. Int Psychogeriatr 1998;10:271-302.

Johnson GV, Jope RS, Binder LI. Proteolysis of tau by calpain. Biochem Biophys Res Commun 1989;163:1505-1511.

Johnson KA, Moran EK, Becker JA, Blacker D, Fischman AJ, Albert MS. Single photon emission computed tomography perfusion differences in mild cognitive impairment. J Neurol Neurosurg Psychiatry 2007;78:240-247.

Kaplan EF, Goodglass H, Weintraub S. The Boston Naming Test. 1991 Boston: Kaplan & Goodglass.

Kawarabayashi T, Younkin LH, Saido TC, Shoji M, Ashe KH, Younkin SG. Age-dependent changes in brain, CSF, and plasma amyloid beta protein in the Tg2576 transgenic mouse model of Alzheimer's disease. J Neurosci 2001;21:372-381.

Kawas CH, Corrada MM, Brookmeyer R, Morrison A, Resnick SM, Zonderman AB, Arenberg D. Visual memory predicts Alzheimer's disease more than a decade before diagnosis. Neurology 2003;60:1089-1093.

Kemppainen NM, Aalto S, Wilson IA, Nagren K, Helin S, Bruck A, Oikonen V, Kailajarvi M, Scheinin M, Viitanen M, Parkkola R, Rinne JO. PET amyloid ligand [11C]PIB uptake is increased in mild cognitive impairment. Neurology 2007;68:1603-1606.

Kemppainen NM, Aalto S, Wilson IA, Nagren K, Helin S, Bruck A, Oikonen V, Kailajarvi

M, Scheinin M, Viitanen M, Parkkola R, Rinne JO. Voxel-based analysis of PET amyloid ligand [11C]PIB uptake in Alzheimer disease. Neurology 2006;67:1575-1580.

Killiany RJ, Gomez-Isla T, Moss M, Kikinis R, Sandor T, Jolesz F, Tanzi R, Jones K, Hyman BT, Albert MS. Use of structural magnetic resonance imaging to predict who will get Alzheimer's disease. Ann Neurol 2000;47:430-439.

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

Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, Holt DP, Bergstrom M, Savitcheva I, Huang GF, Estrada S, Ausen B, Debnath ML, Barletta J, Price JC, Sandell J, Lopresti BJ, Wall A, Koivisto P, Antoni G, Mathis CA, Langstrom B. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. Ann Neurol 2004;55:306-319.

Koivisto K, Reinikainen KJ, Hanninen T, Vanhanen M, Helkala EL, Mykkanen L, Laakso M, Pyorala K, Riekkinen PJ S. Prevalence of age-associated memory impairment in a randomly selected population from eastern Finland. Neurology 1995;45:741-747.

Korf ES, Wahlund LO, Visser PJ, Scheltens P. Medial temporal lobe atrophy on MRI predicts dementia in patients with mild cognitive impairment. Neurology 2004;63:94-100.

Kramer JH, Nelson A, Johnson JK, Yaffe K, Glenn S, Rosen HJ, Miller BL. Multiple cognitive deficits in amnestic mild cognitive impairment. Dement Geriatr Cogn Disord 2006;22:306-311.

Kumar R, Dear KB, Christensen H, Ilschner S, Jorm AF, Meslin C, Rosenman SJ, Sachdev PS. Prevalence of mild cognitive impairment in 60- to 64-year-old community-dwelling individuals: The Personality and Total Health through Life 60+ Study. Dement Geriatr Cogn Disord 2005;19:67-74.

Kurz A, Riemenschneider M, Buch K, Willoch F, Bartenstein P, Muller U, Guder W. Tau protein in cerebrospinal fluid is significantly increased at the earliest clinical stage of Alzheimer disease. Alzheimer Dis Assoc Disord 1998;12:372-377.

Larrieu S, Letenneur L, Orgogozo JM, Fabrigoule C, Amieva H, Le Carret N, Barberger-Gateau P, Dartigues JF. Incidence and outcome of mild cognitive impairment in a population-based prospective cohort. Neurology 2002;59:1594-1599.

Leoni V, Shafaati M, Salomon A, Kivipelto M, Bjorkhem I, Wahlund LO. Are the CSF levels of 24S-hydroxycholesterol a sensitive biomarker for mild cognitive impairment? Neurosci Lett 2006;397:83-87.

Lesne S, Koh MT, Kotilinek L, Kayed R, Glabe CG, Yang A, Gallagher M, Ashe KH. A specific amyloid-beta protein assembly in the brain impairs memory. Nature 2006;440:352-357.

Lewis J, Dickson DW, Lin WL, Chisholm L, Corral A, Jones G, Yen SH, Sahara N, Skipper L, Yager D, Eckman C, Hardy J, Hutton M, McGowan E. Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. Science 2001;293:1487-1491.

Loewenstein DA, Acevedo A, Agron J, Issacson R, Strauman S, Crocco E, Barker WW, Duara R. Cognitive profiles in Alzheimer's disease and in mild cognitive impairment of different etiologies. Dement Geriatr Cogn Disord 2006;21:309-315.

Londos E, Passant U, Gustafson L, Brun A. Neuropathological correlates to clinically defined dementia with Lewy bodies. Int J Geriatr Psychiatry 2001;16:667-679.

Lopez OL, Becker JT, Jagust WJ, Fitzpatrick A, Carlson MC, DeKosky ST, Breitner J, Lyketsos CG, Jones B, Kawas C, Kuller LH. Neuropsychological characteristics of mild cognitive impairment subgroups. J Neurol Neurosurg Psychiatry 2006;77:159-165.

Lopez OL, Jagust WJ, DeKosky ST, Becker JT, Fitzpatrick A, Dulberg C, Breitner J, Lyketsos C, Jones B, Kawas C, Carlson M, Kuller LH. Prevalence and classification of mild cognitive impairment in the Cardiovascular Health Study Cognition Study: part 1. Arch Neurol 2003a;60:1385-1389.

Lopez OL, Jagust WJ, Dulberg C, Becker JT, DeKosky ST, Fitzpatrick A, Breitner J, Lyketsos C, Jones B, Kawas C, Carlson M, Kuller LH. Risk factors for mild cognitive impairment in the Cardiovascular Health Study Cognition Study: part 2. Arch Neurol 2003b;60:1394-1399.

Lyketsos CG, Lopez O, Jones B, Fitzpatrick AL, Breitner J, DeKosky S. Prevalence of neuropsychiatric symptoms in dementia and mild cognitive impairment: results from the cardiovascular health study. JAMA 2002;288:1475-1483.

Manly JJ, Bell-McGinty S, Tang MX, Schupf N, Stern Y, Mayeux R. Implementing diagnostic criteria and estimating frequency of mild cognitive impairment in an urban community. Arch Neurol 2005;62:1739-1746.

Markesbery WR, Schmitt FA, Kryscio RJ, Davis DG, Smith CD, Wekstein DR. Neuropathologic substrate of mild cognitive impairment. Arch Neurol 2006;63:38-46.

Maruyama M, Arai H, Sugita M, Tanji H, Higuchi M, Okamura N, Matsui T, Higuchi S, Matsushita S, Yoshida H, Sasaki H. Cerebrospinal fluid amyloid beta(1-42) levels in the mild cognitive impairment stage of Alzheimer's disease. Exp Neurol 2001;172:433-436.

Maruyama M, Matsui T, Tanji H, Nemoto M, Tomita N, Ootsuki M, Arai H, Sasaki H. Cerebrospinal fluid tau protein and periventricular white matter lesions in patients with mild cognitive impairment: implications for 2 major pathways. Arch Neurol 2004;61:716-720.

Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K. Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc Natl Acad Sci USA 1985;82:4245-4249.

Mayeux R, Honig LS, Tang MX, Manly J, Stern Y, Schupf N, Mehta PD. Plasma A[beta]40 and A[beta]42 and Alzheimer's disease: relation to age, mortality, and risk. Neurology 2003;61:1185-1190.

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939-944.

Mehta PD, Dalton AJ, Mehta SP, Kim KS, Sersen EA, Wisniewski HM. Increased plasma amyloid beta protein 1-42 levels in Down syndrome. Neurosci Lett 1998;241:13-16.

Mehta PD, Pirttila T, Mehta SP, Sersen EA, Aisen PS, Wisniewski HM. Plasma and cerebrospinal fluid levels of amyloid beta proteins 1-40 and 1-42 in Alzheimer disease. Arch Neurol 2000;57:100-105.

Mesulam M. The cholinergic lesion of Alzheimer's disease: pivotal factor or side show? Learn Mem 2004;11:43-49.

Meyding-Lamade U, Ehrhart K, de Ruiz HL, Kehm R, Lamade W. A new technique: serial puncture of the cisterna magna for obtaining cerebrospinal fluid in the mouse--application in a model of herpes simplex virus encephalitis. J Exp Anim Sci 1996;38:77-81.

Meyer J, Xu G, Thornby J, Chowdhury M, Quach M. Longitudinal analysis of abnormal domains comprising mild cognitive impairment (MCI) during aging. J Neurol Sci 2002a;201:19-25.

Meyer JS, Xu G, Thornby J, Chowdhury MH, Quach M. Is mild cognitive impairment prodromal for vascular dementia like Alzheimer's disease? Stroke 2002b;33:1981-1985.

Moir RD, Atwood CS, Romano DM, Laurans MH, Huang X, Bush AI, Smith JD, Tanzi RE. Differential effects of apolipoprotein E isoforms on metal-induced aggregation of A beta using physiological concentrations. Biochemistry 1999;38:4595-4603.

Montine TJ, Kaye JA, Montine KS, McFarland L, Morrow JD, Quinn JF. Cerebrospinal fluid Aβ42, tau and F_2-isoprostane concentrations in patients with Alzheimer's disease, other dementias, and in age.matched controls. Arch Pathol Lab Med 2001;125:510-512).

Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. Neurology 1993;43:2412-2414.

Morris JC, Heyman A, Mohs RC, Hughes JP, van Belle G, Fillenbaum G, Mellits ED, Clark C. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. Neurology 1989;39:1159-1165.

Morris JC, Storandt M, Miller JP, McKeel DW, Price JL, Rubin EH, Berg L. Mild cognitive impairment represents early-stage Alzheimer disease. Arch Neurol 2001;58:397-405.

Mosconi L. Brain glucose metabolism in the early and specific diagnosis of Alzheimer's disease. Eur J Nucl Med Mol Imaging 2005;32:486-510.

Mosconi L, Tsui W, Rusineck H, De Santi S, Li Y, Wang GJ, Pupi A, Fowler J, de Leon M. Quantitation, regional vulnerability, and kinetic modelling of brain glucose metabolism in mild Alzheimer's disease. Eur J Nucl Mol Med Imaging 2007 doi:10.1007/s00259-007-0406-5

Motter R, Vigo-Pelfrey C, Kholodenko D, Barbour R, Johnson-Wood K, Galasko D, Chang L, Miller B, Clark C, Green R. Reduction of beta-amyloid peptide42 in the cerebrospinal fluid of patients with Alzheimer's disease. Ann Neurol 1995;38:643-648.

Nakamura T, Shoji M, Harigaya Y, Watanabe M, Hosoda K, Cheung TT, Shaffer LM, Golde

TE, Younkin LH, Younkin SG. Amyloid beta protein levels in cerebrospinal fluid are elevated in early-onset Alzheimer's disease. Ann Neurol 1994;36:903-911.

Neuropathology Group. Medical Research Council Cognitive Function and Aging Study. Pathological correlates of late-onset dementia in a multicentre, community-based population in England and Wales. Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS). Lancet 2001;357:169-175.

Nilsberth C, Westlind-Danielsson A, Eckman CB, Condron MM, Axelman K, Forsell C, Stenh C, Luthman J, Teplow DB, Younkin SG, Naslund J, Lannfelt L. The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Abeta protofibril formation. Nat Neurosci 2001;4:887-893.

Nordlund A, Rolstad S, Hellstrom P, Sjogren M, Hansen S, Wallin A. The Goteborg MCI study: mild cognitive impairment is a heterogeneous condition. J Neurol Neurosurg Psychiatry 2005;76:1485-1490.

Otto M, Wiltfang J, Tumani H, Zerr I, Lantsch M, Kornhuber J, Weber T, Kretzschmar HA, Poser S. Elevated levels of tau-protein in cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. Neurosci Lett 1997;225:210-212.

Palmer K, Backman L, Winblad B, Fratiglioni L. Detection of Alzheimer's disease and dementia in the preclinical phase: population based cohort study. BMJ 2003;326:245.

Pennanen C, Kivipelto M, Tuomainen S, Hartikainen P, Hanninen T, Laakso MP, Hallikainen M, Vanhanen M, Nissinen A, Helkala EL, Vainio P, Vanninen R, Partanen K, Soininen H. Hippocampus and entorhinal cortex in mild cognitive impairment and early AD. Neurobiol Aging 2004;25:303-310.

Peres K, Chrysostome V, Fabrigoule C, Orgogozo JM, Dartigues JF, Barberger-Gateau P. Restriction in complex activities of daily living in MCI: impact on outcome. Neurology 2006;67:461-466.

Perri R, Serra L, Carlesimo GA, Caltagirone C. Preclinical Dementia: An Italian Multicentre Study on Amnestic Mild Cognitive Impairment. Dement Geriatr Cogn Disord 2007;23:289-300.

Peskind ER, Riekse R, Quinn JF, Kaye J, Clark CM, Farlow MR, Decarli C, Chabal C, Vavrek D, Raskind MA, Galasko D. Safety and acceptability of the research lumbar puncture. Alzheimer Dis Assoc Disord 2005;19:220-225.

Petersen RC, Doody R, Kurz A, Mohs RC, Morris JC, Rabins PV, Ritchie K, Rossor M, Thal L, Winblad B. Current concepts in mild cognitive impairment. Arch Neurol 2001;58:1985-1992.

Petersen RC, Parisi JE, Dickson DW, Johnson KA, Knopman DS, Boeve BF, Jicha GA, Ivnik RJ, Smith GE, Tangalos EG, Braak H, Kokmen E. Neuropathologic features of amnestic mild cognitive impairment. Arch Neurol 2006;63:665-672.

Petersen RC, Smith GE, Ivnik RJ, Tangalos EG, Schaid DJ, Thibodeau SN, Kokmen E, Waring SC, Kurland LT. Apolipoprotein E status as a predictor of the development of Alzheimer's disease in memory-impaired individuals. JAMA 1995;273:1274-1278.

Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. Arch Neurol 1999;56:303-308.

Pirttila T, Kim KS, Mehta PD, Frey H, Wisniewski HM. Soluble amyloid beta-protein in the cerebrospinal fluid from patients with Alzheimer's disease, vascular dementia and controls. J Neurol Sci 1994;127:90-95.

Polvikoski T, Sulkava R, Haltia M, Kainulainen K, Vuorio A, Verkkoniemi A, Niinisto L, Halonen P, Kontula K. Apolipoprotein E, dementia, and cortical deposition of beta-amyloid protein. N Engl J Med 1995;333:1242-1247.

Poorkaj P, Bird TD, Wijsman E, Nemens E, Garruto RM, Anderson L, Andreadis A, Wiederholt WC, Raskind M, Schellenberg GD. Tau is a candidate gene for chromosome 17 frontotemporal dementia. Ann Neurol 1998;43:815-825.

Portet F, Ousset PJ, Visser PJ, Frisoni GB, Nobili F, Scheltens P, Vellas B, Touchon J, MCI Working Group of the European Consortium on Alzheimer's Disease (EADC). Mild cognitive impairment (MCI) in medical practice: a critical review of the concept and new diagnostic procedure. Report of the MCI Working Group of the European Consortium on Alzheimer's Disease. J Neurol Neurosurg Psychiatry 2006;77:714-718.

Price JL and Morris JC. Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. Ann Neurol 1999;45:358-368.

Prince JA, Zetterberg H, Andreasen N, Marcusson J, Blennow K. APOE epsilon4 allele is associated with reduced cerebrospinal fluid levels of Abeta42. Neurology 2004;62:2116-2118.

Rabinovici GD, Furst AJ, O'Neil JP, Racine CA, Mormino EC, Baker SL, Chetty S, Patel P, Pagliaro TA, Klunk WE, Mathis CA, Rosen HJ, Miller BL, Jagust WJ. 11C-PIB PET imaging in Alzheimer disease and frontotemporal lobar degeneration. Neurology 2007;68:1205-1212.

Rasquin SM, Lodder J, Visser PJ, Lousberg R, Verhey FR. Predictive accuracy of MCI subtypes for Alzheimer's disease and vascular dementia in subjects with mild cognitive impairment: a 2-year follow-up study. Dement Geriatr Cogn Disord 2005;19:113-119.

Ravaglia G, Forti P, Maioli F, Martelli M, Servadei L, Brunetti N, Pantieri G, Mariani E. Conversion of mild cognitive impairment to dementia: predictive role of mild cognitive impairment subtypes and vascular risk factors. Dement Geriatr Cogn Disord 2006;21:51-58.

Ready RE, Ott BR, Grace J, Cahn-Weiner DA. Apathy and executive dysfunction in mild cognitive impairment and Alzheimer disease. Am J Geriatr Psychiatry 2003;11:222-228.

Reitan RM. Validity of the trail making test as an indicator of organic brain damage. Percept Mot Skills 1958;8:271-276.

Ribeiro F, de Mendonca A, Guerreiro M. Mild cognitive impairment: deficits in cognitive domains other than memory. Dement Geriatr Cogn Disord 2006;21:284-290.

Riemenschneider M, Buch K, Schmolke M, Kurz A, Guder WG. Cerebrospinal protein tau is elevated in early Alzheimer's disease. Neurosci Lett 1996;212:209-211.

Riemenschneider M, Lautenschlager N, Wagenpfeil S, Diehl J, Drzezga A, Kurz A. Cerebrospinal fluid tau and beta-amyloid 42 proteins identify Alzheimer disease in subjects with mild cognitive impairment. Arch Neurol 2002;59:1729-1734.

Riemenschneider M, Schmolke M, Lautenschlager N, Guder WG, Vanderstichele H, Vanmechelen E, Kurz A. Cerebrospinal beta-amyloid ((1-42)) in early Alzheimer's disease: association with apolipoprotein E genotype and cognitive decline. Neurosci Lett 2000;284:85-88.

Riemenschneider M, Wagenpfeil S, Vanderstichele H, Otto M, Wiltfang J, Kretzschmar H, Vanmechelen E, Forstl H, Kurz A. Phospho-tau/total tau ratio in cerebrospinal fluid discriminates Creutzfeldt-Jakob disease from other dementias. Mol Psychiatry 2003;8:343-347.

Ritchie K, Artero S, Touchon J. Classification criteria for mild cognitive impairment: a population-based validation study. Neurology 2001;56:37-42.

Royall DR, Chiodo LK, Polk MJ. Misclassification is likely in the assessment of mild cognitive impairment. Neuroepidemiology 2004;23:185-191.

Royall DR, Palmer R, Chiodo LK, Polk MJ. Executive control mediates memory's association with change in instrumental activities of daily living: the Freedom House Study. J Am Geriatr Soc 2005;53:11-17.

Russel EW. A multiple scoring method for the assessment of complex memory functions. J Cons Clin Psychol 1975;43:800-809.

Sacuiu S, Sjogren M, Johansson B, Gustafson D, Skoog I. Prodromal cognitive signs of dementia in 85-year-olds using four sources of information. Neurology 2005;65:1894-1900.

Saez-Valero J, Barquero MS, Marcos A, McLean GA, Small DH. Altered glycosylation of acetylcholinesterase in lumbar cerebrospinal fluid of patients with Alzheimer's disease. J Neurol Neurosurg Psychiatry 2000;69:664-667.

Sahlin C, Lord A, Magnusson K, Englund H, Almeida CG, Greengard P, Nyberg F, Gouras GK, Lannfelt L, Nilsson LN. The Arctic Alzheimer mutation favors intracellular amyloid-beta production by making amyloid precursor protein less available to alpha-secretase. J Neurochem 2007;101:854-862.

Saxton J, Lopez OL, Ratcliff G, Dulberg C, Fried LP, Carlson MC, Newman AB, Kuller L. Preclinical Alzheimer disease: neuropsychological test performance 1.5 to 8 years prior to onset. Neurology 2004;63:2341-2347.

Scheltens P, Leys D, Barkhof F, Huglo D, Weinstein HC, Vermersch P, Kuiper M, Steinling M, Wolters EC, Valk J. Atrophy of medial temporal lobes on MRI in "probable" Alzheimer's disease and normal ageing: diagnostic value and neuropsychological correlates. J Neurol Neurosurg Psychiatry 1992;55:967-972.

Schenk D. Amyloid-beta immunotherapy for Alzheimer's disease: the end of the beginning. Nat Rev Neurosci 2002;3:824-828.

Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton

M, Kukull W, Larson E, Levy-Lahad E, Viitanen M, Peskind E, Poorkaj P, Schellenberg G, Tanzi R, Wasco W, Lannfelt L, Selkoe D, Younkin S. Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nat Med 1996;2:864-870.

Schonknecht P, Pantel J, Hartmann T, Werle E, Volkmann M, Essig M, Amann M, Zanabili N, Bardenheuer H, Hunt A, Schroder J. Cerebrospinal fluid tau levels in Alzheimer's disease are elevated when compared with vascular dementia but do not correlate with measures of cerebral atrophy. Psychiatry Res 2003;120:231-238.

Schoonenboom SN, Visser PJ, Mulder C, Lindeboom J, Van Elk EJ, Van Kamp GJ, Scheltens PH. Biomarker profiles and their relation to clinical variables in mild cognitive impairment. Neurocase 2005;11:8-13.

Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. Physiol Rev 2001;81:741-766.

Senior K. Dosing in phase II trial of Alzheimer's vaccine suspended. Lancet Neurol 2002;1:3.

Seubert P, Vigo-Pelfrey C, Esch F, Lee M, Dovey H, Davis D, Sinha S, Schlossmacher M, Whaley J, Swindlehurst C. Isolation and quantification of soluble Alzheimer's beta-peptide from biological fluids. Nature 1992;359:325-327.

Simic G, Kostovic I, Winblad B, Bogdanovic N. Volume and number of neurons of the human hippocampal formation in normal aging and Alzheimer's disease. J Comp Neurol 1997;379:482-494.

Simonsen AH, McGuire J, Hansson O, Zetterberg H, Podust VN, Davies HA, Waldemar G, Minthon L, Blennow K. Novel panel of Cerebrospinal fluid biomarkers for the prediction of progression to Alzheimer dementia in patients with mild cognitive impairment. Arch Neurol 2007;64:366-370.

Sjogren M, Davidsson P, Tullberg M, Minthon L, Wallin A, Wikkelso C, Granerus AK, Vanderstichele H, Vanmechelen E, Blennow K. Both total and phosphorylated tau are increased in Alzheimer's disease. J Neurol Neurosurg Psychiatry 2001;70:624-630.

Skoog I, Davidsson P, Aevarsson O, Vanderstichele H, Vanmechelen E, Blennow K. Cerebrospinal fluid beta-amyloid 42 is reduced before the onset of sporadic dementia: a population-based study in 85-year-olds. Dement Geriatr Cogn Disord 2003;15:169-176.

Smith MJ, Kwok JB, McLean CA, Kril JJ, Broe GA, Nicholson GA, Cappai R, Hallupp M, Cotton RG, Masters CL, Schofield PR, Brooks WS. Variable phenotype of Alzheimer's disease with spastic paraparesis. Ann Neurol 2001;49:125-129.

Solfrizzi V, Panza F, Colacicco AM, D'Introno A, Capurso C, Torres F, Grigoletto F, Maggi S, Del Parigi A, Reiman EM, Caselli RJ, Scafato E, Farchi G, Capurso A, Italian Longitudinal Study on Aging Working Group. Vascular risk factors, incidence of MCI, and rates of progression to dementia. Neurology 2004;63:1882-1891.

Spillantini MG, Murrell JR, Goedert M, Farlow MR, Klug A, Ghetti B. Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. Proc Natl Acad Sci USA 1998;95:7737-7741.

Spires TL and Hyman BT. Transgenic models of Alzheimer's disease: learning from animals. NeuroRx 2005;2:423-437.

Squire LR and Zola-Morgan S. The medial temporal lobe memory system. Science 1991;253:1380-1386.

Stamer K, Vogel R, Thies E, Mandelkow E, Mandelkow EM. Tau blocks traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress. J Cell Biol 2002;156:1051-1063.

Strozyk D, Blennow K, White LR, Launer LJ. CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. Neurology 2003;60:652-656.

Sunderland T, Mirza N, Putnam KT, Linker G, Bhupali D, Durham R, Soares H, Kimmel L, Friedman D, Bergeson J, Csako G, Levy JA, Bartko JJ, Cohen RM. Cerebrospinal fluid beta-amyloid1-42 and tau in control subjects at risk for Alzheimer's disease: the effect of APOE epsilon4 allele. Biol Psychiatry 2004;56:670-676.

Tabert MH, Manly JJ, Liu X, Pelton GH, Rosenblum S, Jacobs M, Zamora D, Goodkind M, Bell K, Stern Y, Devanand DP. Neuropsychological prediction of conversion to Alzheimer disease in patients with mild cognitive impairment. Arch Gen Psychiatry 2006;63:916-924.

Tapiola T, Overmyer M, Lehtovirta M, Helisalmi S, Ramberg J, Alafuzoff I, Riekkinen PS, Soininen H. The level of cerebrospinal fluid tau correlates with neurofibrillary tangles in Alzheimer's disease. Neuroreport 1997;8:3961-3963.

Tapiola T, Pirttila T, Mehta PD, Alafuzofff I, Lehtovirta M, Soininen H. Relationship between apoE genotype and CSF beta-amyloid (1-42) and tau in patients with probable and definite Alzheimer's disease. Neurobiol Aging 2000a;21:735-740.

Tapiola T, Pirttila T, Mikkonen M, Mehta PD, Alafuzoff I, Koivisto K, Soininen H. Three-year follow-up of cerebrospinal fluid tau, beta-amyloid 42 and 40 concentrations in Alzheimer's disease. Neurosci Lett 2000b;280:119-122.

Tervo S, Kivipelto M, Hanninen T, Vanhanen M, Hallikainen M, Mannermaa A, Soininen H. Incidence and risk factors for mild cognitive impairment: a population-based three-year follow-up study of cognitively healthy elderly subjects. Dement Geriatr Cogn Disord 2004;17:196-203.

Tierney MC, Yao C, Kiss A, McDowell I. Neuropsychological tests accurately predict incident Alzheimer disease after 5 and 10 years. Neurology 2005;64:1853-1859.

Tomlinson BE, Blessed G, Roth M. Observations on the brains of non-demented old people. J Neurol Sci 1968;7:331-356.

Tsukamoto K, Watanabe T, Matsushima T, Kinoshita M, Kato H, Hashimoto Y, Kurokawa K, Teramoto T. Determination by PCR-RFLP of apo E genotype in a Japanese population. J Lab Clin Med 1993;121:598-602.

van Gool WA, Kuiper MA, Walstra GJ, Wolters EC, Bolhuis PA. Concentrations of amyloid beta protein in cerebrospinal fluid of patients with Alzheimer's disease. Ann Neurol 1995;37:277-279.

van Groen T, Liu L, Ikonen S, Kadish I. Diffuse amyloid deposition, but not plaque number, is reduced in amyloid precursor protein/presenilin 1 double-transgenic mice by pathway lesions. Neuroscience 2003;119:1185-1197.

Vandermeeren M, Mercken M, Vanmechelen E, Six J, van de Voorde A, Martin JJ, Cras P. Detection of tau proteins in normal and Alzheimer's disease cerebrospinal fluid with a sensitive sandwich enzyme-linked immunosorbent assay. J Neurochem 1993;61:1828-1834.

Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S, Amarante P, Loeloff R, Luo Y, Fisher S, Fuller J, Edenson S, Lile J, Jarosinski MA, Biere AL, Curran E, Burgess T, Louis JC, Collins F, Treanor J, Rogers G, Citron M. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science 1999;286:735-741.

Vigo-Pelfrey C, Seubert P, Barbour R, Blomquist C, Lee M, Lee D, Coria F, Chang L, Miller B, Lieberburg I. Elevation of microtubule-associated protein tau in the cerebrospinal fluid of patients with Alzheimer's disease. Neurology 1995;45:788-793.

Visser PJ, Kester A, Jolles J, Verhey F. Ten-year risk of dementia in subjects with mild cognitive impairment. Neurology 2006;67:1201-1207.

Wahlund LO, Julin P, Johansson SE, Scheltens P. Visual rating and volumetry of the medial temporal lobe on magnetic resonance imaging in dementia: a comparative study. J Neurol Neurosurg Psychiatry 2000;69:630-635.

Waldemar G, Walovitch RC, Andersen AR, Hasselbach SG, Bigelow R, Joseph JL, Paulson OB, Lassen NA. 99mTc-bicisate (neurolite) SPECT brain imaging and cognitive impairment in dementia of the Alzheimer type: a blinded read of image sets from a multicenter SPECT trial. J Cereb Blood Flow Metab 1994;14 Suppl 1:S99-105.

Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature 2002;416:535-539.

Wechsler D. Wechsler memory scale - revised manual. 1987 San Antonio: Psychological Corporation.

Wechsler D. Wechsler adult intelligence scale - revised. 1981 Cleveland: Psychological Corporation.

Wentzel C, Rockwood K, MacKnight C, Hachinski V, Hogan DB, Feldman H, Ostbye T, Wolfson C, Gauthier S, Verreault R, McDowell I. Progression of impairment in patients with vascular cognitive impairment without dementia. Neurology 2001;57:714-716.

Wilson RS, Schneider JA, Barnes LL, Beckett LA, Aggarwal NT, Cochran EJ, Berry-Kravis E, Bach J, Fox JH, Evans DA, Bennett DA. The apolipoprotein E epsilon 4 allele and decline in different cognitive systems during a 6-year period. Arch Neurol 2002;59:1154-1160.

Wimo A, Winblad B, Jönsson J. An estimate of total worldwide societal costs of dementia 2005. Alzheimers Dement 2007 doi:10.1016/j.jalz.2007.02.001.

Winblad B, Palmer K, Kivipelto M, Jelic V, Fratiglioni L, Wahlund LO, Nordberg A,

Backman L, Albert M, Almkvist O, Arai H, Basun H, Blennow K, de Leon M, DeCarli C, Erkinjuntti T, Giacobini E, Graff C, Hardy J, Jack C, Jorm A, Ritchie K, van Duijn C, Visser P, Petersen RC. Mild cognitive impairment--beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. J Intern Med 2004;256:240-246.

Wolfe MS. The gamma-secretase complex: membrane-embedded proteolytic ensemble. Biochemistry 2006;45:7931-7939.

Xu Y, Jack CR,Jr, O'Brien PC, Kokmen E, Smith GE, Ivnik RJ, Boeve BF, Tangalos RG, Petersen RC. Usefulness of MRI measures of entorhinal cortex versus hippocampus in AD. Neurology 2000;54:1760-1767.

Yaffe K, Petersen RC, Lindquist K, Kramer J, Miller B. Subtype of mild cognitive impairment and progression to dementia and death. Dement Geriatr Cogn Disord 2006;22:312-319.

Younkin SG, Ertekin-Taner N, Xin T, Baker M, Younkin LH, Hutton M ,Graff-Radford N. Role of elevated amyloid beta protein in patients with typical late onset Alzheimer's disease. Neurobiol Aging 2000;21 (1S):S136 (abstract 612).

Zetterberg H, Wahlund LO, Blennow K. Cerebrospinal fluid markers for prediction of Alzheimer's disease. Neurosci Lett 2003;352:67-69.