TURUN YLIOPISTON JULKAISUJA
ANNALES UNIVERSITATIS TURKUENSIS

SARJA - SER. D OSA - TOM. 978

MEDICA - ODONTOLOGICA

MILD COGNITIVE IMPAIRMENT AND EARLY DETECTION OF ALZHEIMER`S DISEASE

A Positron Emission Tomography Study

by

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TURUN YLIOPISTO
UNIVERSITY OF TURKU
Turku 2011

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ISBN 978-951-29-4726-3 (PRINT
ISBN 978-951-29-4727-0 (PDF)
ISSN 0355-9483
Painosalama Oy – Turku, Finland 2011

To Nea and Santeri

Cover: [11C] PIB PET images in a normal control subject (left), [11C] PIB-positive normal control, [11C] PIB-negative MCI subject, [11C] PIB-positive MCI subject, highly [11C] PIB-positive MCI subject and a [11C] PIB-positive AD patient (right).

ABSTRACT

Jaana Koivunen

MILD COGNITIVE IMPAIRMENT AND EARLY DETECTION OF ALZHEIMER`S DISEASE

A Positron Emission Tomography Study

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Annales Universitatis Turkuensis Ser D.
Painosalama Oy, Turku, Finland 2011

Alzheimer`s disease (AD) is characterised neuropathologically by the presence of extracellular amyloid plaques, intraneuronal neurofibrillary tangles, and cerebral neuronal loss. The pathological changes in AD are believed to start even decades before clinical symptoms are detectable. AD gradually affects episodic memory, cognition, behaviour and the ability to perform everyday activities. Mild cognitive impairment (MCI) represents a transitional state between normal aging and dementia disorders, especially AD. The predictive accuracy of the current and commonly used MCI criteria devide this disorder into amnestic (aMCI) and non-amnestic (naMCI) MCI. It seems that many individuals with aMCI tend to convert to AD. However many MCI individuals will remain stable and some may even recover. At present, the principal drugs for the treatment of AD provide only symptomatic and palliative benefits. Safe and effective mechanism-based therapies are needed for this devastating neurodegenerative disease of later life. In conjunction with the development of new therapeutic drugs, tools for early detection of AD would be important.

In future one of the challenges will be to detect at an early stage these MCI individuals who will convert to AD. Methods which can predict which MCI subjects will convert to AD will be much more important if the new drug candidates prove to have disease-arresting or even disease–slowing effects. These types of drugs are likely to have the best efficacy if administered in the early or even in the presymptomatic phase of the disease when the synaptic and neuronal loss has not become too widespread.

There is no clinical method to determine with certainly which MCI individuals will progress to AD. However there are several methods which have been suggested as predictors of conversion to AD, *e.g.* increased [^{11}C] PIB uptake, hippocampal atrophy in MRI, low CSF Aβ42 level, high CSF tau-protein level, apolipoprotein E (APOE) ε4 allele and impairment in episodic memory and executive functions. In the present study subjects with MCI appear to have significantly higher [^{11}C] PIB uptake vs healthy elderly in several brain areas including frontal cortex, the posterior cingulate, the parietal and lateral temporal cortices, putamen and caudate. Also results from this PET study indicate that over time, MCI subjects who display increased [^{11}C] PIB uptake appear to be significantly more likely to convert to AD than MCI subjects with negative [^{11}C] PIB retention. Also hippocampal atrophy seems to increase in MCI individuals clearly during the conversion to AD. In this study [^{11}C] PIB uptake increases early and changes relatively little during the AD process whereas there is progressive hippocampal atrophy during the disease. In addition to increased [^{11}C] PIB retention and hippocampal atrophy, the status of APOE ε4 allele might contribute to the conversion from MCI to AD.

Key words: mild cognitive impairment, Alzheimer`s Disease, [^{11}C] PIB PET, hippocampal atrophy, episodic memory, CSF Aβ42, early disease detection

TIIVISTELMÄ

Jaana Koivunen

LIEVÄ KOGNITIIVINEN HEIKENTYMÄ JA ALZHEIMERIN TAUDIN VARHAINEN TOTEAMINEN

Positron Emissio Tomografia tutkimus

Valtakunnallinen PET-keskus, Kliinisen fysiologian ja isotooppilääketieteen oppiaine, Kliininen laitos, Turun yliopisto
Annales Universitatis Turkuensis Ser. D
Painosalama Oy, Turku, Finland 2011

Alzheimerin taudin (AT) tyypillisia neuropatologisia muutoksia ovat solunulkoiset amyloidiplakit, solunsisäiset neurofibrillikimput sekä aivokuoren hermosolujen tuhoutuminen. Todennäköisesti nämä muutokset alkavat jo vuosia ennen taudin kliinisten oireiden ilmaantumista. Lievällä kognitiivisella heikentymällä (MCI) tarkoitetaan normaalien kognitiivisten ikääntymismuutosten sekä dementian, erityisesti AT:n välivaihetta. MCI luokitellaan muistipainotteiseen l. amnestiseen (aMCI) tai ei-muistipainotteiseen l. non-amnestiseen (naMCI) kognitiiviseen häiriöön. Erityisesti juuri amnestinsen muodon on todettu lisäävän AT:n riskiä. Kokonaisuudessaan MCI:n etiologia on erittäin heterogeeninen, eivätkä kaikki sairastu AT:iin. Joidenkin MCI henkilöiden oireet saattavat pysyä vuosikausia muuttumattomina ja osa jopa toipuu.

Tällä hetkellä AT:n lääkehoito painottuu ainoastaan taudin oireiden hallintaan. Ensisijaisen tärkeää olisi kehittää turvallisia ja tehokkaita taudin patologiaan vaikuttavia hoitomuotoja. Yhdessä uusien taudin etenemiseen ja pysäyttämiseen tähtäävien lääketutkimusten lisäksi olisi myös tärkeää löytää keinot varhaisen AT:n toteamiseen. Erityisen tärkeää olisi tunnistaa ne lievästä muistihäiriöstä kärsivät henkilöt, joilla on suurentunut riski sairastua AT:iin. Tämä tulee olemaan erityisen tärkeää mikäli uudet, tutkimuksen kohteena olevat lääkehoidot, hidastavat tai jopa pysäyttävät AT:n etenemisen. Näiden lääkkeiden hyöty ja teho moninkertaistuu, mikäli lääkitys pystytään aloittamaan mahdollisimman varhaisessa taudin vaiheessa, jopa ennen kliinisten oireiden ilmaantumista.

Lievästä muistihäiriöstä kärsivällä henkilöllä saattaa olla lisääntynyt riski sairastua AT:iin, mikäli hänellä todetaan normaalista poikkeavia muutoksia tietyissä tutkimuksissa, esimerkiksi PET-kuvauksessa suurentunut tiettyjen aivokuorialueiden amyloidikertymä, MRI-kuvauksella todettu hippokampuksen atrofia, pienentynyt selkäydinnesteen (CSF) Aβ42 pitoisuus tai suurentunut CSF tau-proteiinipitoisuus. Riskiä lisäävät myös apolipoproteiini E (APOE) ε4 sekä episodisen l. tapahtumamuistin sekä toiminnanohjauksen heikentyminen. Tässä tutkimuksessa todettiin, että [^{11}C] PIB merkkiaineella havaittu lisääntynyt amyloidikertymä voidaan todeta MCI henkilöillä usealla aivokuorialueella terveisiin ikääntyviin henkilöihin verrattuna. Nämä aivokuorialueet ovat frontaalinen, parietaalinen sekä lateraalinen temporaalialue. Suurentuneita kertymiä todettiin lisäksi putamenin sekä posteriorisen cingulumin alueella. Tämän tutkimuksen avulla selvitettiin lisäksi, että PET-kuvauksella havaittu [^{11}C] PIB kertymä lisääntyy merkittävimmin AT:n varhaisessa vaiheessa, kun taas myöhäisemmässä vaiheessa lisääntyminen on vähäistä. Sen sijaan hippocampuksen ja entorinaalisen kuorikerroksen atrofia progredioi tautipatologian edetessä. Myös APOE ε4 alleelin, yksilön perintötekijöissä, todettiin lisäävän MCI henkilön riskiä sairastua AT:iin.

Avainsanat: lievä kognitiivinen heikentymä, Alzheimerin tauti, [^{11}C] PIB PET, hippokampuksen atrofia, episodinen muisti, CSF Aβ42, varhainen taudin tunnistaminen

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ABBREVIATIONS

Aβ	beta amyloid
AD	Alzheimer`s Disease
ADAS	Alzheimer`s Disease Assesment Scale
ADL	activities of daily living
aMCI	amnestic mild cognitive impairment
aMCI-SD	amnestic mild cognitive impairment single domain
aMCI-MD	multidomain amnestic mild cognitive impairment
naMCI-SD	single domain non-amnestic mild cognitive impairment
naMCI-MD	multidomain non-amnestic mild cognitive impairment
APOE	apolipoprotein E
APP	amyloid precursor protein
AutoROI	automated ROI analysis
AUC	area under the curve
[^{11}C] PIB	carbon-11 labeled 2-(4`-methylaminophenyl)-6-hydroxybenzothiazole
CDR	Clinical Dementia Rating
CERAD	Consortium to Establish a Registry for Alzheimer`s Disease
CP	cored plaque
CSF	cerebrospinal fluid
DLB	dementia with Lewy bodies
DP	diffuse amyloid plaque
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders version IV
EDTA	ethylene diamine tetra-acetic acid
ELISA	enzyme-linked immunosorbent assay
[^{18}F] FDG	fluorine-18 labeled fluoro-2-deoxy-D-glucose
fMRI	functional magnetic resonance imaging
FTD	frontotemporal dementia
FTLD	frontotemporal lobe degeneration
GFAP	glial fibrillary acidic protein
IADL	instrumental activities of daily living
MCI	mild cognitive impairment
MMSE	Mini-Mental State Examination
MRI	magnetic resonance imaging
MTL	medial temporal lobe
NFT	neurofibrillary tangle
NINCDS-ADRDA	The National Institute of neurological and Communicative Disorders and Stroke and the Alzheimer`s Disease and Related Disorders Association
NMDA	N-methyl-D-aspartate
NP	neuritic plaque

PCR	polymerase chain reaction
PDD	Parkinson`s Disease dementia
PET	positron emission tomography
PPA	primary progressive aphasia
PS or PSEN	presenilin
rCMRgluc	regional cerebral glucose metabolism
ROC	receiver operating characteristics
ROI	Region Of Interest
SD	standard deviation
SPET	single photon emission tomography
SPM	Statistical Parametric Mapping
SUV	standardized uptake value
T1	longitudinal relaxation
T2	transverse relaxation
VAD	vascular dementia

LIST OF ORIGINAL PUBLICATIONS

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

I Koivunen J, Pirttilä T, Kemppainen N, Aalto S, Herukka S-K, Jauhiainen A.M, Hänninen T, Hallikainen M, Rinne J.O. PET amyloid ligand [^{11}C] PIB uptake and cerebrospinal fluid β-amyloid in mild cognitive impairment. *Dementia and Geriatric Cognitive Disorders* 2008; 26:378-383.

II Okello A, Koivunen J, Edison P, Archer H.A, Turkheimer F.E, Någren K, Bullock R, Walker Z, Kennedy A, Fox N.C, Rossor M.N, Rinne J.O, Brooks D.J. Conversion of amyloid positive and negative MCI to AD over 3 years An [^{11}C] PIB PET study. *Neurology* 2009; 73.10:754-760.

III Koivunen J, Scheinin N, Virta J.R, Alto S, Vahlberg T, Någren K, Helin S, Parkkola R, Viitanen M, Rinne J.O. PET imaging of amyloid deposition in patients with mild cognitive impairment: A two year follow-up study. *Neurology* 2011; 76.12: 1085-90.

IV Koivunen J, Karrasch, M, Scheinin N, Aalto S, Vahlberg T, Någren K, Helin S, Viitanen M, Rinne J.O. Cognitive decline and amyloid accumulation in MCI patients. *Submitted.*

1. INTRODUCTION

Mild cognitive impairment (MCI) is an etiologically heterogenous syndrome characterized by memory performance below the age norm, otherwise unimpaired intellectual functioning, and well preserved activities of daily living. Some MCI individuals will progress to AD, or to some other dementias but some MCI individuals may even recover. The prevalence of MCI among the population >65 years in industrialized countries, is about 10-25 % (Luck et al. 2007). Elderly individuals with MCI constitute a high-risk population of developing dementia, especially AD. The annual conversion rate of MCI to AD has been estimated in general to be about 10-15% (Morris et al. 2001). The most common subtype of MCI is amnestic MCI (aMCI). The incidence of aMCI subtype has been estimated to range between 9.9 and 40.6 per 1,000 person-years, with the incidence of non-amnestic MCI subtypes being between 28 and 36.3 per 1,000 person-years. If considers all forms of MCI, then incidence rates of 51 and 76.8 per 1,000 person-years have been reported (Luck et al. 2007). The typical feature of amnestic MCI is prominent memory impairment, with other cognitive abilities being relatively normal and this form likely progresses to AD (Peterssen and Morris 2005). Other subtypes of MCI have been estimated to be risk at progress to other dementia, i.e. vascular dementia (VAD), dementia with Lewy bodies (DLB) and frontotemporal dementia (FTD) (Petersen 2004). All MCI individuals does not necessarily progress to dementia and may even revert to normal (Anchisi et al. 2005). The most noteworthly risk factors of incident MCI are higher age (Manly et al. 2008, Tervo et al. 2004), lower education (Stern et al. 2006, Hall et al. 2006) and hypertension (Etgen et al. 2009).

The present work assessed the rates of conversion of MCI to AD during a follow-up of two to three years. The objective was also to compare the levels of beta-amyloid deposition with [^{11}C] PIB PET between MCI converters (MCI subjects who later convert to AD) and non-converters. It was considered important to focus on specific biomarkers that wouls predict incipient AD. It was also decided to examine whether [^{11}C] PIB uptake would be more sensitive than the CSF Aβ42 concentration at being able to detect an increased amyloid burden in MCI. Another focus of interest was also to examine the levels of beta-amyloid deposition and the changes in the hippocampal atrophy by magnetic resonance imaging (MRI) during a 2-year follow-up in MCI converters, non-converters and healthy elderly controls. In late AD, individuals suffer severe impairments in all cognitive areas. Individuals with MCI have only mildly impaired performance in an objective neuropsychological test but relatively intact global cognition. The present work assessed also the changes in cognitive functions during a 2-year follow-up period in MCI converters and non-converters.

2. REVIEW OF THE LITERATURE

2.1 Alzheimer`s Disease

Alzheimer`s Disease (AD) is a progressive neurodegenerative disorder associated with disruption of neuronal function, a gradual deterioration in cognition, loss of independence for performing activities of daily living and behavioural changes. AD is the most common cause of dementia, accounting for about 70% of all dementias. The prevalence of AD ranges from 4.4% in persons aged 65 years to 22% at ages 90 and older (Lobo et al. 2000). In global terms, it is estimated that 35.6 million people are currently living with dementia. This number is expected to increase to over 115 million by 2050 (Alzheimer`s Disease International Consortium 2010). The actual number of dementia patients is much higher since a large number of people live with dementia but never receive a clinical diagnosis. These numbers emphasize that dementia is rapidly becoming a major threat to healthcare provision in our societies.

Both genetic and environmental factors contribute to the pathogenesis of AD. In general, two subgroups can be recognized depending on the age at which the first clinical symptoms become apparent; early-onset AD (onset age <65years) and late-onset AD (onset age >65 years). Most patients develop AD at a later age, only about 2-5% represent the familial, early-onset type of the AD (Bettens et al. 2010). Gene mutations have been identified in three different genes which lead to the early-onset form of AD; The amyloid precursor protein gene (APP) and two presenilin genes (PSEN1 and PSEN2).

Sporadic, late-onset AD is a multifactorial disease. The precise etiology of AD is still unknown. There are several well known risk factors for sporadic AD *i.e* age, positive family history, APOE ε4 allele and vascular risk factors (Zigman et al. 1996). Chronic diseases and conditions, such as diabetes, elevated blood cholesterol level, hypertension in midlife, and depression, have been associated with an increased risk for AD (Kivipelto et al. 2008). Furthermore; current smoking and alcohol consumption has been reported to be associated with increased risk for AD (Daviglus et al. 2010). On the other hand , APOE2 allele, possibly intake anti-inflammatory agents and high education have been claimed to decrease the risk of AD (Roe et al. 2007, Kemppainen et al. 2008).

2.1.1 Risk factors of conversion to Alzheimer`s Disease

Mild Cognitive Impairment (MCI), especially the amnestic subtype, has been estimated to be a transitional stage of AD (Morris et al. 2004). MCI is a genetically complex condition and currently there are no major genes known to be involved in MCI. Each of the impairments possibly underlying MCI (such as AD, vascular pathology and depression) may partly have a genetic origin and this is the reason why MCI is genetically so complex. A few studies have indicated that the APOEε4 allele is associated with a greater

likelihood of progression of MCI to AD (Bookheimer and Burggren 2009, Farlow et al. 2004). More studies are needed to determine whether APOE and other possible genes have synergistic interactions with age, gender and gene-enviroment interactions, that would be associated with MCI. With respect to the amnestic MCI subtypes, a significant impact of age on the incidence has been found in a few studies that have analysed age as a possible risk factor (Manly et al. 2008, Tervo et al. 2004). In contrast, no significant impact of gender could be identified as a risk factor.

In some studies a higher level of education (>12 years) has been postulated to be a protective factor for incident amnestic MCI subtypes (Stern 2006, Hall et al. 2006, Tervo et al. 2004). The impact of higher cognitive activity on the incidence of amnestic MCI subtypes has also been identified as a protective factor (Verghese et al. 2006). It is also important to remember that sometimes subjective impairments are the only indication of incipient cognitive deterioration in highly educated persons because of the reserve functions in the cerebral cortex and therefore their results in cognitive tests seem to be similar as in the healthy elderly.

Vascular factors, including hypertension, hyperlipidemia, diabetes mellitus and history of stroke, seem to be risk factors for cognitive decline (Etgen et al. 2009). It has also been suggested that a deficiency of vitamin B12 might contribute to age-associated cognitive impairment (Malouf and Evans 2008). Result from animal studies show that a vitamin B-deficient diet can cause cognitive dysfunction and may be responsible for rarefaction of microvasculature (Troen et al. 2008). Some studies has also found a correlation between low levels of vitamin D in serum and deficits in cognitive function (Oudshoorn et al. 2008). In some animal studies, hyperhomocysteinemia has increased the production of beta-amyloid causing spatial memory deficits that could be attenuated by folate and vitamin-B12 treatment (Zhang et al. 2009). Further studies are needed to confirm these inter-relationships and their relevance to MCI in humans.

A few studies have suggested that estrogen hormones may have neuroprotective effects. Estrogens promote neuronal sprouting, decrease the level of amyloid in cerebral cortex of rats and have some anti-inflammatory properties (Goodman et al. 1996). There are some cross-sectional studies indicating that estrogen treatment in postmenopausal women can result in better cognitive performance (Schmidt et al. 1996, Kampen and Sherwin 1994). The metabolites of testosterone, estradiol or dihydrotestosterone, are known to bind to receptors in memory-relevant regions like hippocampus and amygdala (Janowsky 2006). The association between thyroid dysfunction, hypo- or hyperthyroidism, and cognitive impairment and dementia is well established (Dong et al. 2005). The control of treatable somatic risk factors is of clear relevance in individuals with MCI, particularly as there is still no unequivocal evidence for the efficacy of causal interventions targeting the underlying neurodegenerative disease processes and the drugs used to treat the symptoms when they have become manifest provide only symptomatic relief. Potential risk and protective factors of convertion to AD are summarized in **Table 1.**

Table 1. Potential risk and protection factors for conversion to Alzheimer`s Disease

	risk factors	protective factors
Age	x	
Apolipoprotein E ε4	x	
Apolipoprotein E ε2		x
Education >12 years		x
Cognitive activity		x
Hypertension	x	
Diabetes mellitus	x	
History of stroke	x	
Deficiency of vitamin B12	x	
Low levels of vitamin D	x	
Deficiency of folate	x	
Estrogen hormones		x
Testosterone hormones		x
Thyroid dysfunctions	x	

2.1.2 Clinical course, diagnosis and treatment of Alzheimer`s Disease

AD is a stage concurrent disorder, the clinical symptoms of which correspond to anatomical and temporal evolution of the pathological changes. The course of the AD is characterized by a gradual onset with a progressive decline in cognitive functions. Often the first sign is an impairment in the episodic memory, leading to problems in learning, storing and recalling new information. Later, other cognitive domains become also impared. Gradually the patients start to develop disorders of language and perception, lack of concentration, impairments in executive functions, and problems of temporal and spatial orientation. The typical behavioural symptoms with AD patients are depression, irritability and apathy. As the disease proceeds more behavioural problems may appear, for example, agitation, aggression and hallucinations. In addition, the motor system becomes affected in the severe stages of AD, and many of the patients will exhibit extrapyramidal symptoms, most often rigidity and hypokinesia. At the final stage of the disease, AD patients are unable to feed themselves, become incontinent and with limited speech capabilities. Swallowing and coughing become increasingly disturbed; the most common cause of death in AD patients is pneumonia. The clinical disease duration is approximately 10 years after the first manifestation, but this may vary considerably between the patients. Reasons for this heterogenous disease duration is unknown.

The diagnosis of AD is nowadays based on clinical picture which can be supported by magnetic resonance imaging (MRI) and certain cerebrospinal fluid biomarkers. The potential tools for detecting incipient AD are demonstrated in **Table 2.** A substantial decline in episodic memory and executive function typically occurs at the onset of AD (as reported by the patient or an informant). It is possible to document these cognitive impairments with neuropsychological tests. Structural brain imaging, MRI, can support the diagnosis of AD by revealing atrophy in hippocampus and other medial temporal

lobe structures. Hippocampal atrophy is seen in normal aging as well, but is greatly accelerated and steadily progressive in AD (Jack et al. 2000, Petersen et al. 2000). Hippocampal and entorhinal atrophy shows a strong correlation with cognitive decline and AD pathologic markers such as neuronal and neurofibrillary tangle counts and Braak and Braak pathological staging (Fleischman et al. 2005, Schonheit, Zarski, and Ohm 2004). The entorhinal cortex and the hippocampus have also been found to be significantly atrophied in individuals with MCI and mild AD in comparison to control individuals. However, some MCI subjects has no atrophy and findings in brain structures are similar like in normal aging (Fan et al. 2008). There is some indication that the extent of hippocampal atrophy can predict which MCI subjects will progress to AD. With MRI it might also be possible to detect atrophic changes in cognitively normal older people predicting the future development of cognitive impairment (Sanchez-Benavides et al. 2010, Chetelat and Baron 2003).

As well as hippocampal area, the entorhinal cortex is now well established as being important in episodic memory (Squire and Zolamorgan 1991). Several studies have noted that hippocampal and entorhinal atrophy correlate well with the severity of memory impairment, especially for delayed recall measures. A number of studies in MCI have found a positive correlation between hippocampal volume and memory performance (Stoub et al. 2006, Wolf et al. 2004).

There is no single test or biomarker that could with certainty predict incipient AD (Nestor, Scheltens, and Hodges 2004). Today the clinical diagnosis of AD and MCI, as mentioned above, largely depends on clinical criteria (3.1.2 and 3.2.3). Hence it is difficult to distinguish with certainly between MCI and normal aging. Several biomarkers have been tested for their ability to predict the conversion from MCI to AD. There are some promising results about certain CSF biomarkers. The two biomarkers for which there is the most conclusive data are β-amyloid 1-42 (Aβ42) and the protein tau, which reflect extracellular senile plaque and intracellular neurofibrillary tangles, respectively. Increased CSF levels of total tau (T-tau) have been suggested to be a marker of the intensity of neuronal damage and degeneration while increased levels of CSF tau phosphorylated at threonine 181 (P-tau) and decreased CSF Aβ42 concentrations are claimed to be markers for the degenerative processes (Hansson et al. 2006, Andreasen and Blennow 2005, Strozyk et al. 2003). In conjunction with other diagnostic tools, Aβ42, T-tau and P-tau separately or in combination are claimed to be able to discriminate individuals with AD and MCI from nondemented aging. There is also evidence, that the CSF Aβ42/tau ratio holds promise as an antecedent biomarker that can predict future dementia in cognitively normal older adults (Fagan et al. 2007).

Follow-up studies have shown that AD-type CSF profile in individuals with MCI strongly predicts further conversion to dementia, with a sensitivity exceeding 80% in a multi-center study (Mattsson et al. 2009) and reaching 95% in a single-.center study of (Hansson et al. 2006), the relative risk of progression to AD being 17.7 times higher in those MCI individuals who had pathological concentrations of T-tau and Aβ42 at

baseline. The mean levels of CSF β-amyloid42 (Aβ42) typically decrease in AD whereas the levels of CSF tau and phospho-tau increase (Blennow and Hampel 2003). A significant correlation has been observed between the levels of CSF Aβ42 and the numbers of amyloid plaques in AD patients. The exact connection between these two parameters is still not known. The tentative time course of Aβ-plaques and Aβ42 in CSF in AD evolution might be that the increasing amyloid burden in cerebral cortex decreases the concentration of Aβ42 in the CSF (Nordberg 2010).

Many studies has documented decreased regional cerebral uptake and metabolism of glucose in AD. In AD research, [^{18}F] FDG, fluorine-18 labelled derivative of glucose, fluorodeoxyglucose, is the most commonly employed PET tracer. Its uptake reflects the activity of glucose metabolism in the brain, and indirectly neuronal and synaptic function. In AD, [^{18}F] FDG-PET typically reveals reduced glucose metabolism predominantly in the parietal and superior/posterior temporal regions, posterior cingulate and the precuneus (Li et al. 2008, Mosconi et al. 2005, Kantarci et al. 2004, Herholz et al. 2003).

The stage and severity of AD can be evaluated with Clinical Dementia Rating (CDR) scale, which rates cognitive and functional performance on a scale ranging from 0 to 3. With higher scores indicating a greater severity of impairment (Morris 1997). Functional status can also be evaluated with the General Disorder (GDS) FAST scale.

It can be difficult to distinguish between normal aging and the early stages of AD. It is not easy to determine when patients have reached the very early stage of disease, particularly because it is likely that a preclinical stage of AD exists in which senile plaques, neuritic plaques, and neurofibrillary tangles occur in sufficient numbers to meet standard neuropathogical criteria for AD in the absence of overt symptoms or signs of dementia (Price et al. 2009). Recently, new research criteria for the clinical diagnosis of AD have been suggested (Dubois et al. 2007). Early and significant episodic memory impairment is the central factor of these new criteria. In addition to progressive memory impairment, there must be at least one or more abnormal biomarkers *i.e.* evidence of damage in the structural neuroimaging with MRI, molecular neuroimaging with PET, and CSF analysis of Aβ or tau proteins. However, validation studies in existing and prospective cohorts are still needed to refine these criteria. Furthermore, other causes of memory impairment must also be considered. In some cases, laboratory testing and brain imaging can be used to rule out other or concomitant causes of cognitive impairment such as cerebrovascular disease, hydrocephalus, hypothyroidism, vitamin B12 deficiency, central nervous system infection, a cognitive disorder related to human immunodeficiency virus infection, adverse effects of prescribed medications, substance abuse and brain tumors.

[^{11}C] PIB, Pittsburgh Compound B, N-methyl-2-(4`-methylaminophenyl)-6-hydroxybenzothiazole, is a thioflavin T derivative which binds selectively to amyloid plaques (Kemppainen et al. 2006, Klunk et al. 2004). Several subsequent [^{11}C] PIB PET studies have confirmed that patients with AD show significantly greater [^{11}C] PIB uptake

compared with healthy controls in the neocortical brain regions typically affected by Aβ accumulation (Kemppainen et al. 2006, Price et al. 2005).

Table 2. The potential tools for predicting incipient AD

Decline in episodic memory and executive function
The atrophy of hippocampus and entorhinal cortex (MRI)
Decreased cerebrospinal Aβ42 protein
Increased cerebrospinal TAU protein
Decreased regional cerebral uptake and metabolism of glucose ([^{18}F] FDG)
Increased number of regional cerebral amyloid plaques ([^{11}C] PIB)

At present, the principal drugs approved for the treatment of AD are cholinesterase inhibitors (donepezil, rivastigmine and galantamine) as well as the N-methyl-D-aspartate receptor antagonist memantine. These therapies are considered to be symptomatic, palliative interventions. Since at present symptomatic treatments are available, safe and effective mechanism-based therapies that could slow down or halve the disease causes are needed for this devastating neurodegenerative disease of later life.

The experimental support for the "amyloid cascade hypothesis" has lead investigators to identify and evaluate targets for anti-amyloid therapeutic strategies, which are designed to reduce the amyloid burden in the brains of individuals with AD (Tanzi and Bertram 2005). Antibodies directed against the amyloid β reduce cerebral amyloid-β load in transgenic mice (Schroeter et al. 2008) and might be able to block the synaptotoxic effects of amyloid-β oligomers (Shankar et al. 2008). The soluble oligomeric Aβ blocks the synaptic plasticity required for memory formation in the hippocampus, where neuronal loss is seen as the disease progresses. These findings have lead to the hypothesis that the specific inhibition of small molecules evoking the Aβ aggregation, might provide an attractive therapeutic approach for targeting the underlying disease progression of AD at an early stage. One of the central pathological hallmarks of AD is also the aggregation and accumulation of the microtubule-associated tau protein. These hypotheses have stimulated research that has investigated tau protein as a target for AD treatment. The amyloid-β load can be measured by using Positron Emission Tomography (PET) and the radiotracer carbon-11-labelled Pittsburgh compound B ([^{11}C] PIB) (Scheinin et al. 2009, Ikonomovic et al. 2008). With [^{11}C] PIB PET, there have been encouraging findings that a specific amyloid-β antibody (Bapineuzumab) might bind to amyloid β in the brain and facilitate its clearance (Rinne et al. 2010). In order to be really efficacious, treatments that aim to halt or slow down the progression of AD should be ideally started very early, probably during the presymptomatic phase of the disease. One of the aims of the new putative research criteria for AD (Dubois et al. 2007) is to permit the diagnosis of AD earlier than with the currently used clinical criteria.

2.1.3 Neuropathology

The pathological changes in AD may start even decades before clinical symptoms are detectable (Price and Morris 1999). If one considers MCI to represent a transitional stage of AD, then it is clearly important to understand better the etiology of AD and MCI. With that understanding it could be possible to identify markers for the early identification of individuals with prodromal AD at a pre-dementia stage when potential disease-modifying therapies would be most likely to be efficacious and cost-effective.

There are three prominent neuropathological changes that are associated with AD: the neurofibrillary tangles (NFTs), extracellular amyloid deposits and neuronal loss. NFTs consist of paired helical filaments (PHFs) containing the microtubule-associated protein tau. Extracellular amyloid deposits are generally divided into diffuse or neuritic senile plaques which contain the Aβ peptide (Braak and Braak 1991). Amyloid plaques are densely packed, extracellular deposits formed from insoluble Aβ peptides that are cleaved from amyloid precursor protein (APP). The Aβ peptides which contain 42 amino acids are the most susceptible forms to aggregate. The load of amyloid plaques in cerebral cortex and the level of Aβ42 in the brain are central to the pathogenesis of AD and correlate with the cognitive decline observed in AD (Fagan et al. 2006, Hardy and Selkoe 2002, Hardy 1997). It has been postulated that soluble Aβ forms such as monomers, dimers or oligomers are the toxic precursor species of β-amyloid (Selkoe 2008). Aβ plaques appear to start in the neocortex, especially in the regions of the basal neocortex. With time, plaques can be found in the nuclei of the midbrain and in the basal cholinergic nuclei of the forebrain. Finally, the plaques have spread to all of the basal nuclei and cortical regions. In the very late stages of AD, Aβ plaques can be found even in the cerebellum (Braak and Braak 1991). In contrast, intracellular NFTs first appear in the entorhinal regions of the hippocampus and then become more widespread. Over time, there is a widespread loss of neurons and synapses (Braak and Braak 1991). Chronic inflammation probably also plays an important role in the heterogeneous pathogenesis of AD. Activated astrocytes expressing glial fibrillary acidic protein (GFAP) are closely associated with AD pathology, such as tangles, neuritic plaques and amyloid deposits. Beta-amyloid plaques contain both microglia and astrocytes (Korolainen et al. 2005), see **Figure 1.**

Post-mortem studies in MCI have revealed varying degrees of AD pathology. Most individuals with MCI do not meet the neuropathologic criteria for AD, but their pathologic findings point to a transitional state of envolving AD. The most distinct findings involve medial temporal lobe structures. When visually assessed, medial temporal atrophy, especially involving the hippocampus, can been seen. Histopathological findings in post-mortem brain samples of aMCI subjects include the presence of diffuse amyloid plaques (DP), cored plaques (CP) and neuritic plaques (NP). Although most often it is the diffuse plaques which are seen, especially in the neocortex. In general, patients with AD had more CPs and NPs whereas the amyloid burden of individuals with MCI resembled more that of healthy eldery. Neurofibrillary tangles (NFT) can also been seen typically in the entorhinal

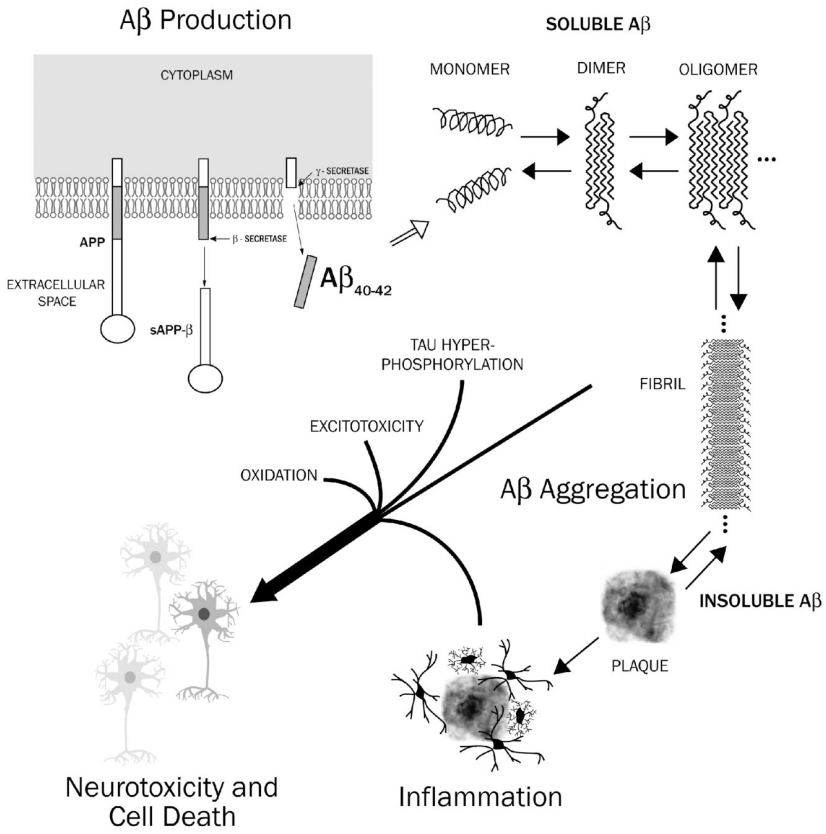


Figure 1. Schematic figure of the production of Aβ (1-40) and Aβ (1-42) peptides from amyloid precursor protein (APP) via sequential β-and γ-secretase cleavages. The Aβ (1-40) and Aβ (1-42) monomers are believed to aggregate in the extracellular space to form soluble Aβ species (oligomers). Some Aβ oligomers are believed to form proto-fibrils, which then form slightly soluble Aβ fibrils and plaques. Oligomers and Aβ-containing fibrils and plaques are believed to set in motion the subsequent damaging processes, such as tau hyperphosphorylation and neurofibrillary tangle formation, generation of excitotoxic species, oxidative damage, neuroinflammation, enlargement of axons dendrites with deposits of hyperphosphorylated tau filaments (dystrophic neuritis), loss of synaptic junctions, and neuronal cell death (Korolainen et al. 2005).

cortex and hippocampal areas. These neurofibrillary changes seemed to be intermediate between the changes of normal aging and those of very early AD. It is possible that there are two main histopathologic findings concerning MCI subjects: diffuse amylod burden in the neocortex and frequent NFTs in medial temporal lobe structures. Other non-specific pathologic features seem to be argyrophilic grain disease, hippocampal sclerosis and vascular lesions (Markesbery et al. 2006, Petersen et al. 2006).

The role of the Aβ-protein in the brain is not entirely clear, but it has been assumed that the soluble forms may cause synaptic dysfunction (Selkoe 2008, Nordberg 2008). The amount of extracellular soluble Aβ in the brain is a better predictor of cognitive impairment in AD than the number of plaques themselves (Nordberg 2008). The fragments of Aβ-protein are snipped from amyloid precursor proteins (APP). One common view is that

in a normal-functioning brain, these protein fragments are broken down and eliminated, but in AD, the problem is that the fragments are not metabolized and thus accumulate in the brain to form plaques. Another common view is that the plaques result from an overproduction of Aβ or APP and this initiates the cytotoxic cascade.

It is relatively common, especially in elderly individuals, to have "mixed" or concomitant pathologies, such as AD-type changes and vascular lesions or AD-type changes associated with Lewy body pathology. It has been estimated that in elderly individuals, the combination of AD-type and vascular pathologies is more common that pure AD-type pathology.

2.2 Mild Cognitive Impairment

Mild Cognitive Impairment (MCI) is a clinical construct that describes individuals with subjective memory complaints and mildly impaired performance on objective neuropsychological tests but relatively intact global cognition and daily functioning (Petersen et al. 1999). According to recent epidemiological data, the prevalence of MCI among the population >65 years in industrialized countries is as high as 10-25% (Luck et al. 2007). Etiology of MCI is very heterogenous. Some individuals with MCI will progress to dementia, some will remain stable and some will even recover. The conversion rate from MCI into dementia is estimated to be around 5-10% annually (Petersen et al. 2001). MCI is believed to represent a transitional state between normal aging and dementia disorders, especially AD, see **Figure 2** .

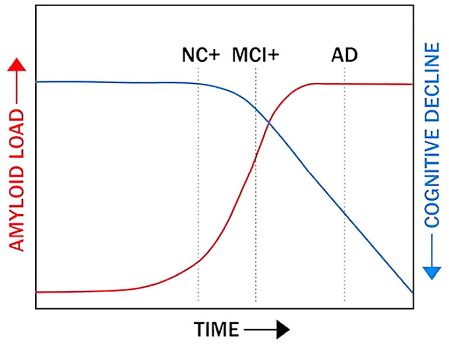


Figure 2. The pathological changes in AD are thought to start even decades before clinical symptoms are detectable. One of the most salient changes in AD and MCI is the β-amyloid burden (Mathis et al. 2007).

NC+=normal cognition, MCI+=mild cognitive impairment, AD+=Alzheimer`s Disease

MCI is commonly divided into four subtypes: amnestic MCI (aMCI-SD), multidomain amnestic MCI (aMCI-MD) (=memory and other domain(s) impaired), multidomain non-amnestic MCI (naMCI-MD) and single domain non-amnestic MCI (naMCI-SD). In particular, the amnestic forms of MCI are risk factors for AD (Petersen and Morris

2005). It has suggested that aMCI-MD with vascular etiology could be the risk factor of vascular dementia (VAD), naMCI-MD with degenerative etiology could lead to dementia with Lewy bodies (DLB), naMCI-MD with vascular etiology might be the risk factor of VAD and naMCI-SD (e.g. language and visuospatial impairments) could lead to frontotemporal dementia (FTD) or DLB (Petersen 2004).

In most studies the annual conversion rate of MCI to AD, as mentioned early, has been 10-15% (Morris et al.2001). Some studies have even reported conversion rates as high as 30-40% (Geslani et al. 2005). Petersen et al. 1999 conducted a detailed characterization of a sample of carefully diagnosed subjects, contrasted the MCI cognitive and clinical findings with those of healthy individuals and AD patients, and reported from follow-up data, that about 50% of the MCI individuals converted to meet the formal criteria for AD within 4 years (Petersen et al. 1999).

2.2.1 Mild cognitive impairment in relation to normal aging

Normal aging is associated with a measurable decline in certain neural and cognitive systems and some resulting behavioural changes. These declines include a decreased speed of information processing, reduced working memory capacity, and impaired long-term memory function. At the same time, knowledge structures remain relatively intact with age (Park 2002). It is very important to understand the precise neural mechanism that determine cognitive outcome in late adulthood as well as being able to identify markers of less successful cognitive aging as early in life as possible.

Increased amyloid deposition is a characteristic feature of individuals with MCI. It is also present in many normal older people, even as many as one third of healthy older adults have been estimated to show significant amyloid deposition (Rodrigue, Kennedy, and Park 2009, Jack 2008). However, β-amyloid deposition is observed in MCI at a higher level than normal older adults. There is considerable evidence to suggest that β-amyloid deposition precedes the decline in cognition and it may be the initiator of a cascade of events that is ultimately responsible for the cognitive decline. Increased amyloid deposition is a strong predictive factor in the conversion to AD.

Aβ-accumulation is believed to be a critical initiating event in a cascade of events that ultimately leads to cognitive decline (Rodrigue, Kennedy, and Park 2009, Nordberg 2008). Since amyloid deposition may well be the first event in this negative cascade in many healthy old adults, amyloid plaques can be found although the individuals behave within normal cognitive limits. One explanation for this might be that the pathology has yet to be expressed behaviourally because this is an early neuropathological event that has its detrimental effects via functional and structural degradation. This may be because the brain responds to the initial pathology by reorganizing and compensating functionally for the amyloid deposition in an attempt to maintain normal cognitive performance. It is well known, that higher levels of education and cognitive activity are protective factors against memory impairments (Kemppainen et al. 2008, Stern 2006, Hall et al. 2006, Tervo et al. 2004).

2.2.2 Diagnosis and clinical features of mild cognitive impairment

There is no agreement in the field on a single set of criteria for MCI. In general, the concept of MCI refers to a group of individuals who have some cognitive impairment but it is not of sufficient severity to constitute dementia. An early and accurate diagnosis of AD is important so that patients and their families can plan for the future when the patient is still able to contribute to decision-making. In addition, therapy can be initiated when overall function may be relatively good.

The clinical symptoms depend on the subtype of the MCI. In amnestic MCI, the main symptom is episodic memory impairment, whereas activities of daily living are well preserved. In nonamnestic MCI, other cognitive functions, *e.g.* language or visuospatial functions, are impaired (Winblad et al. 2004). The generally acknowledged criteria for the amnestic form of MCI are 1): presence of a memory complaint, preferably corroborated by an informant, 2) normal general cognitive functioning, 3) normal activities of daily living, 4) memory impairment in relation to age and education, 5) no dementia (Petersen et al. 2001). Other types of MCI also exist. One of these is MCI with multiple domains slightly impaired, in which nondemented persons exhibit deficits in multiple areas of cognitive functioning. Another form of MCI is single non-memory MCI, in which subjects have deficits in some cognitive domain other than memory. One of these cognitive domains could be a pronounced language disturbance, which could progress to primary progressive aphasia (Petersen et al. 2001).

Nowadays the diagnosis of MCI is made in conjunction with clinical examinations including laboratory analysis, physical examination, neuropsychological tests and often with the help of MRI-scanning. Clinical methods incorporating informant interviews can accurately identify the subset of MCI individuals with prodromal AD (this subset largely corresponds to aMCI). The neurobiological phenotype of aMCI closely resembles that of clinically diagnosed AD, although at a milder stage. Common features include neuropsychiatric symptoms, especially depression (Feldman et al. 2004), over-representation of the APOE ε4 allele (Farlow et al. 2004), volumetric loss in the entorhinal cortex and hippocampus as measured by MRI (Korf et al. 2004), hypometabolism in AD-typical regions as measured by [^{18}F] FDG PET (see chapter 3.1.1), neuronal loss in the entorhinal cortex and hippocampus (Jicha et al. 2006), increased brain markers of oxidative stress (Pratico et al. 2002), cell cycle changes and neuronal cell death (Yang, Mufson, and Herrup 2003) and abnormalities in the cholinenergic nervous system (DeKosky 2008). Several studies have also documented AD-resembling amyloid plaques in several cortical brain regions. It is possible that this amyloid burden can be detected with [^{11}C] PIB PET (Nazri et al. 2010, Li et al. 2008, Forsberg et al. 2008). The latest consensus of AD diagnosis criteria include the symptom of aMCI confirmed by increased [^{11}C] PIB uptake, medial temporal lobe atrophy or abnormal CSF Aβ42, TAU and fTAU (Dubois et al. 2007).

2.2.3 Cognitive and neuropsychological tests in mild cognitive impairment

The cognitive decline can be identified with neuropsychological tests. The most commonly used screening tests are MMSE and CERAD, and subsequently, a more comprehensive neuropsychological examination can be performed. Neuropsychological measures usually reveal an impairment in delayed recall tasks that assess episodic memory and category fluency tasks (e.g naming animals) that require semantic memory and also executive function (Modrego and Ferrandez 2004). MMSE represents a potential screening tool because it is widely used and has been proven to reliably detect cognitive impairment, although it is relatively insensitive. The test battery consists of items assessing orientation, memory, organization and problem solving. The score ranges from 30 (no impairment) to 0 (complete impairment). Only 6 points (out of the maximum 30) are given from memory tasks: 3 from immediate recall and 3 from delayed recall. Subjects with MCI and mild AD score on average 18-30, most often in MCI the score is in the range 25-30 (Feldman and Woodward 2005, Folstein et al. 1975). The CERAD test reveals more detailed information about the severity of cognitive functions. It consists items of verbal fluency, naming, word learning, recall and recognition, symbol digit and symbol recall and MMSE. The CERAD battery of tests provides valuable information about orientation and concentration impairment. Highly educated persons can complete the test questions without difficulties, but nonetheless concurrently be exhibiting incipient cognitive impairments (McLaughlin et al. 2010). Therefore, in more educated individuals or in very mild cases, a detailed neuropsychological investigation is required.

The memory systems are in general classified into five different categories: 1) procedural memory, 2) perceptual representation system, 3) semantic memory, 4) working memory and 5) episodic memory (Tulving 1972). In both AD and MCI subjects, impaired episodic memory is a core feature (Dubois et al. 2007; Petersen 2004). Episodic memory contains a few domains *i.e.* free recall, cued recall, source recall and recognition of actions and short sentences, free recall of words, recognition of names and faces, recall of activities during the test session, and prospective memory (Nilsson, Bäckman, Erngrund, et al.1997). The severity of the deficit in episodic memory, especially verbal memory, has been found to be quite pronounced in aMCI (Jack et al. 1999, Petersen et al. 1994). Episodic memory tends to decrease also with advancing age. It has been proposed, that cognitively unimpaired elderly and early-stage AD patients differ mostly in their ability to acquire and retain new information (Rubin et al. 1998, Ritchie et al. 1997). It is hypothesised that impaired episodic memory in AD and MCI results from the dysfunction of an integrated network that includes the medial temporal lobe, mamillary bodies, dorsomesial thalamus, posterior cingulate, and the connecting white matter tracts (Nestor et al. 2004). Correlations between episodic memory and elements of the limbic-diencephalic network have been detected across the spectrum of MCI and AD but also with healthy aging. It has been noted, that impaired verbal episodic memory is associated with reduced hippocampal volume using structural MRI (Choo et al. 2010, Jack et al. 2009, Leube et al. 2008).

The stage of the disease can be evaluated on the CDR scale, which evaluates cognitive and functional performance (Morris 1997). The CDR scale evaluates subjects on their memory, orientation, judgement and problem solving, community affairs, home and hobbies and personal care based on interviews conducted with both the subject and a caregiver. In MCI, the score usually is either 0 or 0.5 (Morris 1997). MCI individuals has quite normal activity of daily living (ADL) but it has been shown that more complex instrumental activities of daily living (IADL) may already be impaired in the early stages of cognitive decline (Jefferson et al. 2008, Artero et al. 2006, Tuokko et al. 2005). In fact, impairments in IADL have been found to be a strong predictor of progression to dementia in subjects with MCI (Tabert et al. 2002; Peres et al. 2006).

2.3 Positron emission tomography (PET) imaging in mild cognitive impairment

2.3.1 Principles of PET

PET is a practically non-invasive nuclear medicine technique that exploits short lived positron-emitting isotopes to provide quantitative cross-sectional images. The PET technique is widely used in scientific research and clinical medicine. It enables quantification of several biochemical and physiological functions in living organism in vivo by administering pharmaceutical compounds or biological substrates labelled with positron-emitting isotopes. All the isotopes used in this technique are relatively short-lived (half-life for 11C ~20 min, 18F ~110 min and 15O ~ 2 min).

The physical principle of PET is based on annihilation. PET radioisotopes have an excess of protons in their nucleus and a stable state is reached by emission of positron, which is the antimatter equivalent of the electron. Once emitted, the positron, a positively charged beta-particle, travels a distance of a few millimetres, depending on the radioisotope and the density of the tissue (with 11C the average distance 0.56mm, maximum distance 4.1 mm). The emitting positron expends most of its kinetic energy and is terminated in an interaction with a nearby electron, leading to its annihilation. In this annihilation, the masses of positron and electron are converted in to two 511 keV photons that depart the annihilation site in opposite directions ($180^{\circ} \pm 0.25$).

The PET scanner has a ring of detectors surrounding the subject. It registers the two coincidental photons arriving at opposing detectors simultaneously (within nanoseconds) and a decay event is thus recorded. Detection of this coincidence events implies that the annihilation occurred somewhere along the line between the two detectors. The average distance which a positron travels before the annihilation, sets a limit on the absolute spatial resolution of PET. The resolution depends on the size of the detector crystal and the spacing. The ring diameter also affects the resolution, but in addition it is influenced by physical factors such as the distance the positron travels, the noncollinearity of photons and characteristics of the isotope used. The PET images can be disturbed by

factors like scatter, attenuation, accidental coincidences, dead time and partial volume effects. The use of the transmission scan with an external positron emission source or with computerized tomography in PET / CT devices and mathematical corrections can help to correct for these distorting factors. Spatial resolution in the PET image is quite low, on average 3 mm. For example in an MRI image the spatial resolution is clearly higher, 0.2-1 mm.

Single-photon emission tomography (SPET) is a tomographic imaging technique that uses gamma-emitting isotopes instead of positron-emitting isotopes. The technique is similar to conventional nuclear medicine imaging with a gamma camera, with the important difference that SPET can produce three-dimensional information. One advantage of SPET over the PET technique is that the employed radioisotopes have a relatively long half-time so that expensive on-site cyclotron is not needed. Thus SPET is generally less expensive and more available than PET. The spatial resolution of SPET (5-10 mm), however, is poorer than that of the PET technique.

In brain studies, PET allows the *in vivo* assessment of regional blood flow and blood volume, oxygen and glucose metabolism as well as estimating the functioning of several neurotransmitter systems. Today it is also possible to measure a few pathophysiological changes withing the brain such as amyloid plaque and neurofibrillary tangles and microglial activation. In memory disorders, PET technique and PET imaging have become significant supplemental devices, especially in AD and MCI research.

2.3.2 PET radiotracers in mild cognitive impairment research

The radiotracer, which is most extensively used in AD and MCI research is [^{18}F] FDG, *i.e.* fluorine-18 labelled derivative of glucose, fluorodeoxyglucose. Its uptake reflects glucose metabolism in the brain tissue and thus indirectly neuronal and synaptic function. In addition to research use of the [^{18}F] FDG technique, it is widely used in the clinic; [^{18}F] FDG PET can increase diagnostic confidence when one is evaluating the type of dementia in an individual patient. Since different dementia syndromes exhibit different patterns of brain hypometabolism (Klein et al. 2010, Diehl-Schmid et al. 2007).

In AD, [^{18}F] FDG PET reveals reduced glucose metabolism predominantly in the parietal and superior/posterior temporal regions, posterior cingulate and the precuneus (Mosconi et al. 2009, Herholz 2003). It also appears to have prognostic value in presymptomatic AD two or more years before the full clinical manifestation of dementia (Chetelat et al. 2003). In addition, posterior cingulate and medial temporal lobe hypometabolism have been reported in MCI subjects in comparison to normal elderly (De Santi et al. 2001, de Leon et al. 2001). These findings in MCI individuals also predict further conversion to AD. [^{18}F] FDG PET studies can also characterize typical patterns of cerebral hypometabolism in probable AD and aMCI (Clerici et al. 2009, Lowe et al. 2009).

There are new Aβ-specific PET radiotracers which allow quantitative analysis of the Aβ burden *in vivo*. This is important not only for the diagnosis of AD and MCI,

but also for antiamyloid drug development and research. The best validated of these radiotracers is N-methyl-2-(4`-methylaminophenyl)-6-hydroxybenzothiazole, *i.e.* 6-OH-BTA-1; `Pittsburgh Compound-B`([^{11}C] PIB), a carbon-11-labelled derivative of the thioflavin-T amyloid dye. The first studies with [^{11}C] PIB in humans were performed in Uppsala, Sweden in 2002. The PIB compound was developed by William Klunk and Chester Mathis at the Pittsburgh University. The first PET PIB imaging studies were performed in a collaboration between Pittsburgh University, USA, Karolinska Institutet and Uppsala University, Sweden. The initial studies showed significantly higher PIB retention in the frontal, temporal, parietal and occipital cortices and the striatum (1.9-1.5 times differences) in mild AD patients when compared to healthy controls (Klunk et al. 2004).

[^{11}C] PIB binds with very high affinity and high specificity to neuritic Aβ-plaques (Klunk et al. 2004, Klunk et al. 2003). Two *in vivo*-post-mortem correlation studies have been reported for [^{11}C] PIB and both revealed the excellent correspondence between the *in vivo* and post-mortem measures of Aβ (Ikonomovic et al. 2008, Bacskai et al. 2007). In addition, there is known to be a good correlation between frontal cortical brain biopsy β-amyloid load and *in vivo* [^{11}C] PIB retention in the same area in patients with suspected normal pressure hydrocephalus (Leinonen et al. 2008). The molecular structure of [^{11}C] PIB is shown in **Figure 3.**

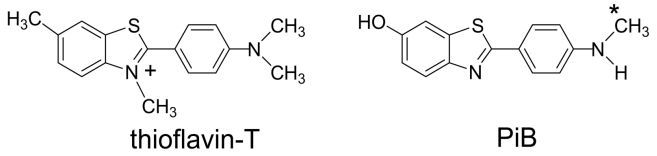


Figure 3. Structures of thioflavin-T and a neutral, lipophilic derivative of thioflavin-T, PIB. The site of radiolabelling with ^{11}C is indicated with an asterisk (*).

From the viewpoint of radiation safety, the use of [^{11}C] PIB in clinical PET studies are is not problematic (Scheinin et al. 2007). 500 MBq seems to be a sufficient dose to visualize Aβ plaques in cerebral cortex. A 500 MBq-injection of [^{11}C] PIB, would result in a radiation dose of 2.37 mSv. Such a dose allows repeated scans in individual subjects. As a comparison, a computed X-ray tomography scan of the body results in a radiation dose of about 10 mSv.

One could predict that amyloid imaging would have potential value for detecting early AD and also in the development and efficacy assessment of new therapies. Therefore, research into the amyloid radiotracers is dynamic. Several amyloid-binding tracers have recently shown potential for clinical applications; [^{18}F] AV-138 (Wey et al. 2009), [^{18}F] flutemetamol (Vandenberghe et al. 2010), [^{18}F] AV-45 (Liu et al. 2010), [^{18}F] BAY94-9172 (Rowe et al. 2008) and [^{11}C] AZD2184 (Johnsson et al. 2009).

In addition, one recently developed radiotracer, 2-(1-{6-[(2-[^{18}F] fluoroethyl)(methyl) amino]-2-naphthyl}ethylidene)malononitrile ([^{18}F] FDDNP), binds to both amyloid plaques and neurofibrillary tangles (Agdeppa et al. 2001). Thus, with [^{18}F] FDDNP, it is possible to evaluate the composite distribution of amyloid and tau pathologies in patients with AD and MCI. However, the problem with [^{18}F] FDDNP is its relatively low signal-to-noise ratio. [^{18}F] FDDNP has been successfully used to monitor disease progression, and it possessed good sensitivity to detect the transformation of MCI subjets into AD (Liu et al. 2007).

In addition to PET radiotracers, one SPET tracer commonly used in dementia research is [^{99m}Tc] HMPAO, hexamethylpropyleneamine oxime. [^{99m}Tc] HMPAO distribution in SPET represents blood flow and can compete with [^{18}F] FDG PET scanning of the brain *e.g.* in dementia research (Kato et al. 2008). [^{99m}Tc] ECD SPET tracer has also been used in an attempt to diagnose dementia and to predict which MCI individuals are likely to convert to dementia (Caroli et al. 2007). One SPET tracer which has been used in amyloid SPET imaging is [^{123}I] IMPY (Newberg et al. 2006).

There are several ligands which can be used to study the functioning of different neurotransmitter systems, such as the acetylcholine, dopamine and serotonin systems. Post-mortem and *in vivo* studies have revealed, that there are impairmenst in several neurotransmitter systems in MCI and AD as well as in other neurodegenerative diseases. Thus, the changes in neurotransmitter systems are not specific for any particular disease or syndrome, and therefore cannot be used in the early diagnosis. However, imaging of various neurotransmitter systems is still useful for instance in studies of the pathophysiology of these diseases and in the development of drug treatment (Nordberg et al. 2010, Rinne et al. 2010).

2.4 [^{11}C] PIB PET

2.4.1 [^{11}C] PIB uptake in normal aging

Histopathologic studies have revealed extensive cortical β-amyloid (Aβ) deposition in AD, but it may also be found in asymptomatic elderly persons as well**.** Asymptomatic cortical Aβ deposition in elderly individuals is well documented with one-fourth or more of the non-demented population age over 75 years having moderate numbers of neuritic plaques in the cerebral cortex (Bennett et al. 2006, Price and Morris 1999). On autopsy, it has been observed that neuropsychologically normal, healthy older adults can exhibit significant neuropathology in the form of amyloid deposition (Dickson et al. 1992). Amyloid imaging with [^{11}C] PIB in normal elderly individuals undergoes uptake into the same regions that show the earliest amyloid deposition in autopsy studies (Braak and Braak 1997), see **Figure 4**.

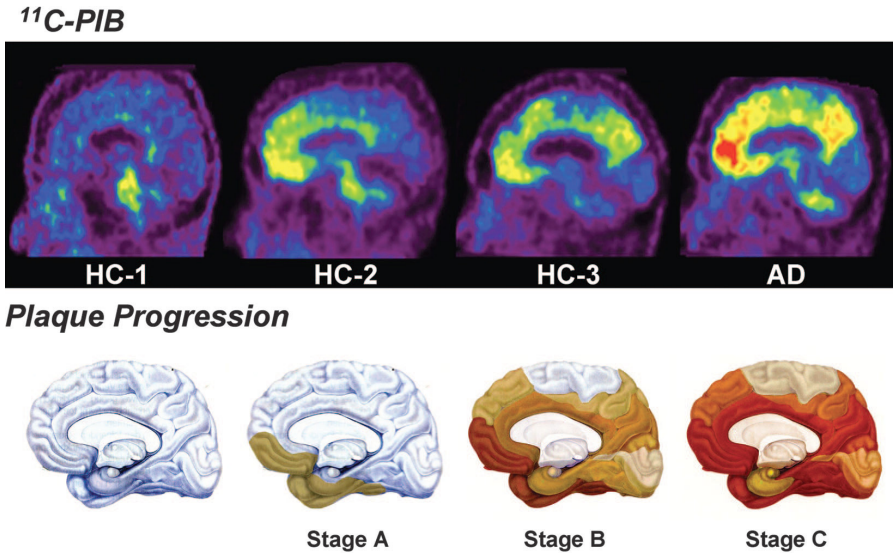


Figure 4. Representative sagittal PET images showing the regional uptake of [^{11}C] PIB, reflecting the Aβ burden in three non-demented healthy subjects (HC 1 to 3) brain and in one patient with AD,the schematics illustrate the stages of Aβ deposition in the human brain as proposed by Braak and Braak (bottom) (Braak and Braak 1997) (from Rowe et al. 2007).

At present, one of the challenges in cognitive neuroscience of aging research is pinpointing the precise neural mechanisms that determine cognitive outcome in late adulthood as well as identifying markers of less successful cognitive aging as early as possible. New imaging tracer ligands offer possibilities for measuring the Aβ burden in the brain and for studying the time course of its progression in nondemented individuals as well as in AD and MCI. The pattern of PIB binding is as well rather similar to AD and diffusely involves prefrontal cortex, lateral and medial parietal regions, lateral temporal cortex and striatum. However, binding patterns can be more focal, and there are a number of studies indicating some preferential uptake in prefrontal cortex and posterior cingulate / precuneus (Villemagne et al. 2008, Rowe et al. 2007, Mintun et al. 2006).

2.4.2 [^{11}C] PIB uptake in mild cognitive impairment

Many studies has shown that [^{11}C] PIB uptake is evidently higher in cortical brain regions (frontal, precuneus, parietal and lateral temporal cortices), when compared to the healthy elderly and AD (Scheinin et al. 2009, Rowe et al. 2007, Kemppainen et al. 2006, Klunk et al. 2004). Similar findings have been reported also with MCI individuals compared to healthy controls (Jack et al. 2008, Kemppainen et al. 2007). It has also been found that episodic memory impairment and increased PIB binding not only displayed a strong correlation in AD patients, but this was also apparent in aMCI subjects (Pike et al. 2007).

Increased [^{11}C] PIB uptake predicts the conversion to AD in MCI subjects (Forsberg et al. 2008, Pike et al. 2007). At the same time, up to 40% of individuals meeting the clinical criteria for MCI do not develop overt clinical dementia (Busse, Angermeyer,

and Riedel-Heller 2006). The amnestic form of MCI might best predict of conversion to AD, as mentioned earlier (see **Figure 5**). It has been found, that there was a strong relationship between impaired episodic memory performance and [^{11}C] PIB binding, both in MCI individuals and healthy ageing subjects (Pike et al. 2007). This relationship was weaker in AD patients and less robust for non-memory cognitive domains. There was also relationship with ApoE ε4 allele and PIB binding; 80% of MCI subjects with a PIB-positive scan carried an ApoE ε4 allele, compared to only 23% of those with a PIB-negative scan (Pike et al. 2007).

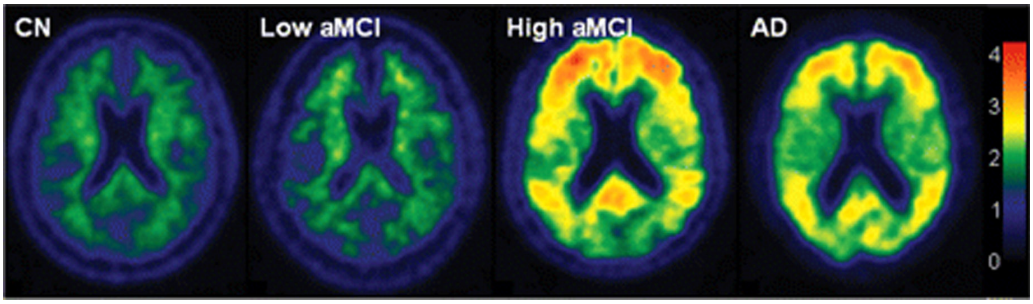


Figure 5. represents [^{11}C] PIB accumulation in normal controls (CN), low PIB ratio amnestic MCI patients (low aMCI), high PIB ratio amnestic MCI patients (high aMCI) and AD patients. The colour scale bar represents the cortical voxel-to-cerebellar retention ratio (Jack et al. 2008).

Many PIB studies in MCI patients have shown [^{11}C] PIB retention as being intermediate between AD and controls, as mentioned early, the distribution being almost bimodal: some individuals showing high [^{11}C] PIB retention and some having [^{11}C] PIB uptake within the control range (Forsberg et al. 2008, Kemppainen et al. 2007, Rowe et al. 2007, Price et al. 2005,). Increased [^{11}C] PIB uptake is seen in the frontal, parietal and lateral temporal cortices, as well as in the posterior cingulate as compared to the healthy elderly (Forsberg et al. 2008, Kemppainen et al. 2007). During follow-up, some of MCI subjects will convert to AD (converters), while some will remain as MCI (non-converters) (Jack et al. 2008). Those MCI subjects who converted to AD during the follow-up period showed higher PIB retention compared to non-converters. However, longer follow-up and a larger number of participants will be needed to conclude whether [^{11}C] PIB PET can indeed discriminate between those MCI individuals who will develop AD and those who will not.

2.4.3 [^{11}C] PIB PET in mild cognitive impairment with relation to CSF biomarkers, MRI and [^{18}F] FDG PET findings

In summary, there is great interest in finding diagnostic tools which could detect an increased risk of on MCI subjects to develop AD. Both structural and functional neuroimaging, as well as CSF Aβ1-42 and Tau, have shown promising results in improving the prediction of which MCI subjects will convert to AD, as mentioned above (2.1.1). The findings indicate that changes in glucose metabolism, as measured by [^{18}F]

FDG PET, might have some predictive value in the detection of MCI individuals at risk of developing AD (Chetelat et al. 2003, Drzezga et al. 2003).

MRI is used in the assessment of brain atrophy and in the evaluation of hippocampus, entorhinal and temporal neocortical volumes. It has significant value to discriminate MCI subjects at risk of developing AD (Chetelat and Baron 2003). Structural MRI can capture disease-related structural changes in the brain by measuring loss of brain volume which is a direct result of loss of neurons, synapses, and supporting cellular structures. Antemortem rates of brain atrophy correlate with neurofibrillary tangle density and rates of ventricular expansion correlate with both plaque and tangle density at autopsy (Silbert et al. 2003). An excellent correlation can be found between hippocampal volume measures obtained on either antemortem MRI (Zarow et al. 2005) or post-mortem MRI (Bobinski et al. 2000) and hippocampal neuron cell counts in autopsy specimens. It has been reported that the correlation between imaging and functional performance follows the expected pattern *i.e.* high PIB retention correlates with greater hippocampal atrophy (Jack et al. 2008). With MRI, it is possible to identify which subjects with MCI or AD are at higher risk to decline more rapidly than others (Vemuri et al. 2009).

Many studies show higher mean [^{11}C] PIB retention among MCI individuals than in age-matched healthy controls, but lower retention than in AD patients, as mentioned earlier (see 2.4.2). In AD, [^{18}F] FDG PET has revealed reduced glucose metabolism predominantly in the parietal and superior/posterior temporal regions, as well as in the posterior cingulate and the precuneus (see 2.3.2). In MCI subjects, there is heterogeneity in the changes of cerebral glucose metabolism (Anchisi et al. 2005). Some studies have shown that only 50% of MCI cases have reduced [^{18}F] FDG uptake (Mosconi et al. 2005, Drzezga et al. 2005, Chetelat et al. 2003). Only a few studies have found consistent abnormalities when particular risk areas are specifically evaluated, such as the mesial temporal lobes (Mosconi 2005, Mosconi et al. 2004, de Leon et al. 2001). One possible explanation for the different results may be differences in subject populations in the various studies and in the heterogenic nature of MCI. It has been found that the MCI group has a higher cerebral glucose metabolism when compared to AD patients (Forsberg et al. 2008). This could represent a compensatory increase in glucose metabolism at a very early stage of neurodegeneration. One reason for these partly conflicting research findings might be that the initiation of amyloid accumulation first induces compensatory mechanisms in the brain tissue and these may include hypermetabolism in the neurons. It has found recently that [^{18}F] FDG was superior in the classification of controls versus MCI subjects, whereas PIB and FDG were similar in their ability to classify AD (Li et al. 2008).

Cerebrospinal fluid biomarkers, such as Aβ 1-42, Tau and pTau might be useful in identifying MCI subjects at risk of developing AD, as mentioned earlier (2.1.1) (Herukka et al. 2005, Blennow and Hampel 2003). A combination of Aβ 1-42 and Tau displayed sensitivity as high as 95% and specificity of 83% in detecting MCI subjects that developed AD (Hansson et al. 2006). However, [^{11}C] PIB PET imaging might be more sensitive than CSF biomarkers in its ability to discriminate prodomal AD patients (Forsberg et al. 2008).

3. OBJECTIVES OF THE STUDY

I To compare the sensitivities of [^{11}C] PIB uptake and CSF Aβ42 concentration in detecting the increased amyloid burden in MCI.

II To investigate the rates of conversion of MCI to AD during a 3-year follow-up period and to compare the levels of amyloid deposition between MCI converters and nonconverters. To assess whether PIB-positive subjects with MCI are significantly more likely to convert to AD than PIB-negative patients and whether faster converters have higher PIB retention levels at baseline than slower converters.

III To compare the levels of Aβ deposition during a 2-year follow-up period in MCI converters, nonconverters and healthy elderly controls and to investigate hippocampal atrophy and [^{11}C] PIB retention changes when conversion to AD has occured.

IV To examine the relationship between baseline [^{11}C] PIB uptake and the cognitive decline during a two-year follow-up in MCI individuals.

4. SUBJECTS AND METHODS

4.1 Subjects

All MCI subjects fulfilled the criteria of MCI proposed by Petersen et al. 2001 (see 2.2.2). All subjects had memory impairment and possible mild decline in other cognitive domains. The CDR scale in MCI participants was 0.5. Mild changes in ADL were allowed, but no subjects had dementia at baseline. The conversion to AD was diagnosed when the patient fulfilled the DSM-IV criteria for dementia and the NINCS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer`s Disease and Related Disorders Association) criteria for probable AD. All participants underwent a standardized clinical examination and neuropsychological test battery assessing attention, memory, language, psychomotor function and visuoconstructive abilities. The studies were approved by the local ethical committee and all participants provided their informed consent.

4.1.1 Patients and controls (I)

The patients group (n=15; 6 women, 9 men) in study **I** consisted of subjects enrolled in the Department of Neurology of the University of Turku and Department of Neurology of the Kuopio University Hospital. The mean age of the subjects was 71.1 years (SD 7.2). The MCI subjects were follow-up for 2 years, after which 6 patients (40%) had converted to AD. The control subjects (22; 16 women, 6 men) were healthy volunteers, and had no history of neurological or psychiatric disease. The mean age of this group was 69.0 years (SD 6.9).

4.1.2 Patients and controls (II)

The patient group in study **II** consisted of 31 MCI subjects. Fourteen of these subjects with MCI (mean age 66.6 years: SD 9.6) were recruited from UK hospitals (Imperial Collage Healthcare NHS Trust [London], The National Hospital for Neurology and Neurosurgery [London], St. Margaret`s Hospital [Epping, and Victoria Hospital [Swindon]). The remaining 17 subjects (mean age 71.7 years: SD 5.3) were recruited from the University Hospital of Turku, Finland. The majority of MCI subjects were newly diagnosed cases. All of them underwent a neurologic examination , neuropsychological assessment, and routine blood analysis. The criteria for MCI participants were not applied for the definition of objective memory impairment. The appropriate age-matched normative values were used when determining evidence for objective memory impairment on performance on tests of objective memory including word list saving (%) of the Consortium to Establish a Registry for Alzheimer`s Disease (CERAD) (Morris et al. 1988) test battery, Wechsler Memory Scale-Reviced (WM-R) (Wechsler 1987) (Turku), Alzheimer`s Disease Assessment Scale (ADAS) word list learning with delayed

recall (Rosen et al. 1984), California Verbal Learning Test (CVLT) (Delis et al. 1987), and immediate and delayed recall of a modified complex figure (Becker et al. 1987) (London). The control group consisted of 26 healthy volunteers without any history or evidence of neurologic or psychiatric disease. Fourteen of the healthy volunteers were recruited from London, UK hospitals and their mean age was 64.6 years; SD 6.3. Twelve healthy control subjects were recruited From Turku, University Hospital; mean age 66.2 years, SD 6.8. All participants gave informed consent, and the study was approved by the Ethics Committees of Hammersmith and Queen Charlotte`s Hospitals and the Southwest Finland Health Care District.

4.1.3 Patients and controls (III)

The patients in study **III** were recruited from the University Hospital of Turku, Finland. Altogether 29 subjects (10 women, 19 men; mean age 73.9, SD 6.8 years) with MCI were studied. At the baseline of the study, none of the subjects had dementia. Seventeen of these MCI participants converted to AD during the approximately 2 year follow-up period. The control subject group consisted of 13 healthy volunteers; 7 women and 6 men. The mean age in this group was 70.6 years (SD 5.6). They had no history or evidence of neurological or psychiatric diseases.

4.1.4 Patients (IV)

Sixteen subjects with MCI were studied; 6 women and 10 men. Their mean age was 73.7 years (SD 6.6). Nine of these MCI participants converted to AD during the approximately 2 year follow-up period. At baseline, all subjects underwent neuropsychological tests including CERAD subtests: word fluency, naming, word list learning, word list saving (%), word list delayed recall, clock drawing, trail making A and B and Stroop test. All sixteen subjects underwent the 2 year follow-up testing.

4.2 Methods

4.2.1 Neuropsychological assessments

In all studies **I-IV**, the neuropsychological test battery included tests for episodic and semantic memory, attention, language and also visuospatial and visuoconstructive abilities. Tests in study **I, III** and **IV** were performed by trained personnel, either a certified psychologist or advanced students of psychology supervised by an experienced neuropsychologist. The testing was performed at Turku PET Centre, in the Clinical Research Services Turku, or at Åbo Akademi University facilities. In study **II,** 28 of participants were tested in London, UK. The appropriate age-matched normative values were used when determining evidence for objective memory impairment on performance on tests of objective memory including word list saving (%) of the CERAD (Morris et al. 1988) test battery, Wechsler Memory Scale-Reviced (WM-R) (Wechsler, D 1987) (Turku), Alzheimer`s Disease Assessment Scale (ADAS) word list learning with delayed

recall (Rosen et al. 1984), California Verbal Learning Test (CVLT) (Delis, D.C. et al 1987), and immediate and delayed recall of a modified complex figure (Becker et al. 1987)(London). If the individual had a subjective memory complaint, scored at least 1.5 SD under the mean reference value in any memory domain, the general cognitive function was normal, and the activities of daily living were preserved, the criteria for MCI were fulfilled (Petersen et al. 2001). If the individual showed widespread impairment (scored at least 1.5 SD under the mean reference value in any other cognitive domain in addition to the memory deficit), and had a progressive decline of memory and other cognitive functions over time, then the NINCDS-ADRDA criteria for probable AD were fulfilled. In study **IV,** at baseline 16 participants underwent neuropsychological tests assessing word fluency, naming, word list learning, word list saving (%), word list delayed recall, clock drawing, trail making A and B and Stroop test. All MCI subjects underwent the 2 year follow-up testing. The MMSE was carried out in all studies (**I-IV).**

4.2.2 MRI

All of the participants in studies **I-IV** underwent T1-weighted volumetric brain MRI to allow structure-function coregistration with [^{11}C]-PIB PET images. The voxel size of the T1-weighted 3-dimensional scan was 0.50 x 0.50 x 1.00 mm. Additionally, T2-weighted MRI was performed to exclude the possibility that structural changes were not associated with subject`s clinical diagnosis of MCI or any neurological disease (healthy controls). The MRI scanning was performed in PET Centre Turku with a Philips Gyroscan Intera 1.5 T scanner (Philips, Best, The Netherlands). In study **III** hippocampal atrophy was also visually evaluated by an experienced neuroradiologist, who was blinded to the clinical status of the subjects. The MRI scans were pooled into groups with various degrees of atrophy, resulting in different scores (0, 1, 2, 3, 4). Score 0 represents no atrophy, and score 4 severe atrophy(see **Table 3**).

Table 3. Visual rating of medial temporal lobe atrophy

Score	Width of choroid fissure	Width of temporal horn	Height of hippocampal formation
0	**N**	**N**	**N**
1	↑	**N**	**N**
2	↑↑	↑	↓
3	↑↑↑	↑↑	↓↓
4	↑↑↑	↑↑↑	↓↓↓

↑ = increase, ↓ = decrease, N = normal.
(Scheltens et al. 1992)

4.2.3 CSF biomarker analyses

In study **I,** a CSF sample was taken from the MCI subjects. A sample was collected by lumbar puncture into polypropylene tubes and stored at -70◦C until analysis. The CSF

levels of Aβ42, totalTAU and pTAU (^{181}P) were measured with a commercial ELISA (Innogenetics, Ghent, Belgium) according to the manufacturer`s protocol. The ELISA analyses were done blinded to the diagnosis. The cut-off values of CSF biomarkers used are based on control material of the analysing laboratory (Herukka et al 2005). Study baseline CSF levels of Aβ42, totalTAU and pTAU from 46 control and 78 subjects with MCI were measured. Twenty-three of these MCI participants developed dementia during the study. Abnormal biomarkers were found early in the course of AD. The cut-off values of the biomarkers were (in pg/ml): Aβ42<450, totalTAU >400, pTAU>70, Aβ42/pTAU<6.5. In study **I**, the [^{11}C] PIB PET scans was performed within 12 months of the CSF sampling in all subjects. The control subjects did not undergo CSF sampling.

4.2.4 APOE ε4 status

In studies **I-III,** the APOE ε4 statuses of the study participants were determined. In study **II**, APOE status was available in 17 of 31 MCI subjects. A sample was collected by vena puncture. Samples was take to EDTA tubes and stored at -20 degrees until analysis. The APOE ε4 status was measured by PCR, Restriction Enzyme Analysis and agarose gel electrophoresis (Tsukamoto et al. 1993).

4.2.5 PET imaging

PET examination in studies **I, III** and **IV** were performed with a GE Advance PET scanner (General Electric Medical Systems, Milwaukee, Wisconsin, USA), using the three-dimensional scanning mode (septa retracted). This three-dimensional scanning mode makes it possible to have 35 slices of 4.25 mm thickness covering the whole brain. The spatial resolution (full width at half-maximum) of the camera was 4.3 mm transaxially and 4.3 mm axially. Prior to the injection of [^{11}C] PIB, a 8-minute transmission scan was performed to measure tissue attenuation of 511 keV γ-radiation. The acquired imaging data was mathematically corrected for attenuation, scatter, accidental coincidence and dead time, and reconstructed for analyses. The imaging data was reconstructed into a 128 x 128 matrix using a transaxial Hann filter with a 4.6mm cut-off and an axial ramp filter with an 8.5mm cut-off. In study **II,** 28 participants (London) were scanned at the Cyclotron Building, Hammersmith Hospital, using a Siemens ECAT EXACT HR+ camera in 3-dimensional acquisition mode with an axial field of view of 15.5 cm. Sixty-three transaxial image planes were displayed as 2.46mm slices with a reconstructed axial resolution of 5.4mm and a transaxial resolution of 5.6mm. The transmission scan time in London was 10 minutes. Before the scans, subjects were positioned in the scanner with three-dimensional laser alignment with reference to the orbito-meatal line. A 90-min dynamic PET imaging was executed using [^{11}C] PIB as a tracer. The mean injected activity for subjects in Turku was 468 MBq (SD 68). The specific radioactivity of the [^{11}C] PIB at the time of administration was 29.0 MBq/ nmol (mean ~3μg). In London, the mean injected dose of [^{11}C] PIB for patients was 367 MBq (SD 25). The [^{11}C] PIB tracer was administered intravenously as a rapid bolus injection.

4.2.6 Quantitative analysis of [^{11}C] PIB uptake

Brain [^{11}C] PIB uptake was analysed with automated ROI analysis in all studies **I-IV**. In ROI analysis, a summated image of the last 30 minutes of the emission scan was used, because this time period (60 to 90 min post-injection) was deemed to best illustrate specific tracer uptake. Automated ROI analysis was performed using standardized ROIs defined on the MRI template image representing brain anatomy in accordance with MNI space (Montreal Neurological Institute database). In order to ensure a common stereotaxic space, the mean images of 12 spatially normalized MRIs were used instead of a single MRI image. Since this method is based on a common stereotaxic space, i.e. spatially normalized images, the operator induced error in defining ROIs individually for each subject can be avoided. The ROIs were defined using Imadeus software (version 1.50; Forima, Turku, Finland) bilaterally on the anterior and posterior cingulated, lateral frontal cortex, caudate nucleus, putamen, thalamus, lateral temporal cortex, parietal cortex, medial temporal lobe, white matter and pons. In addition, a neocortical score was calculated by averaging [^{11}C] PIB uptake values in the lateral frontal cortex, lateral temporal cortex, occipital cortex, parietal cortex and posterior cingulate in studies **I** and **IV.** The average regional ratio values of [^{11}C] PIB uptake were calculated using these ROIs from spatially normalized parametric images. Spatial normalization of parametric images was performed using a ligand specific [^{11}C] PIB template, which was created from fourteen [^{11}C] PIB scans. Parametric images represented [^{11}C] PIB uptake in each pixel were calculated as a region-to-cerebellum ratio of the radioactivity concentration over 60-90 min, the cerebellar ROI being drawn on cerebellar cortex of spatially normalized MRI template images. Individual MRIs were coregistered to summated [^{11}C] PIB images and then MRIs were normalized using a T1-weighted MRI template. In study **II**, ROI analysis with 28 participants in London, was made by using an in-house probabilistic brain atlas (Hammers et al. 2003). The probabilistic brain atlas allows anatomical labelling of the results of group studies in stereotaxic space, automated anatomical labelling of individual brain imaging datasets, and the statistical assessment of normal ranges for structure volumes and extents. The high resolution MRI images were corrected for nonuniformity and reoriented along both the anterior-posterior commissure (AC-PC) line horizontally and the midsagittal plane sagittally.

4.2.7 Statistical analysis

Voxel-wise statistical analysis of [^{11}C] PIB uptake was done with Statistical Parametric Mapping (SPM) analyses, performed with Statistical Parametric Mapping (Friston et al. 1995) software version 2 (SPM2) and Matlab 6.5 for Windows (Math Works, Natick, MA, USA) in study **III.** Prior to the analysis of the parametric images; the spatially normalized images where the [^{11}C] PIB signal in each voxel had been divided by the cerebellar [^{11}C] PIB signal of the same individual; were smoothed with 4 mm full-width at half maximum (FWMH) Gaussian kernel. Group differences in baseline [^{11}C] PIB uptake were analyzed with 1-way analysis of variance and, for the subjects, a split-plot design was used for a repeated measures 2-way analysis of variance with the scan

(baseline or follow-up) as the condition term. Similarly as in the statistical analysis of regional [^{11}C] PIB uptake, voxel-wise statistical inferences were done with t-contrasts for the group differences at baseline and within-group differences between baseline and follow-up. Cluster-level Family-Wise Error (FWE) corrected p-values below 0.01 were regarded as significant and the results localized with the MNI Space utility (http://www.ihb.spb.ru/~pet lab/MSU/MSUMain.html). In study **II,** the normalized [^{11}C] PIB ratio images were smoothed in London using a Gaussian kernel of 6 mm. Between-group comparisons were made using a voxel threshold of $p<0.0001$ (converters vs nonconverters) and $p<0.01$ (faster converters vs slower and non-converters), with an extent threshold of 200 voxels.

The reproducibility at both the region and voxel level method has been found to range from good to excellent (Aalto et al. 2009). An automated analysis method based on an efficient scanning protocol provides reproducible results for [^{11}C] PIB uptake and appears to be suitable for PET studies aimed at the quantitation of amyloid accumulation in the brain of AD patients for the evaluation of progression and treatment effects.

In studies **I- IV**, statistical analyses were done by SAS System for Windows, release 9.2 (SAS Institute, Gary, NC, USA). In study **II**, SPSS for Windows 14.0 statistical software, was used to analyze the results of 28 participants in London, UK. In study **I**, a 2-sample t test was performed to compare the [^{11}C] PIB uptake values between MCI subjects and controls. Pearson`s correlation coefficients were calculated between [^{11}C] PIB uptake and CSF Aβ42 levels. In addition to the region-to-cerebellum ratio method in study **I**, the distribution volume ratio (DVR) was also calculated (Logan et al 1996). The mean +2 SD value was considered as representing increased (abnormal) [^{11}C] PIB uptake. This value was determined for the two brain regions (posterior cingulate and frontal cortex) showing the most robust differences between AD patients and healthy controls. The cut-off values were 1.57 for the posterior cingulate and 1.29 for the frontal cortex. ROC (receiver operating characteristic) analysis was also performed to compare the [^{11}C] PIB uptake values in different brain areas between MCI subjects and controls (study **I**). In study **III**, the difference in the hippocampal atrophy scores between converters, non-converters and controls were tested using Kruskal-Wallis test and Mann-Whitney U-test with Bonferroni correction. The changes in atrophy scores within groups were tested with Wilcoxon Signed Rank test. Correlations were calculated using Pearson correlation coefficients. P-values less than 0.05 were statistically significant. The differences in the [^{11}C] PIB uptake and MMSE values between the groups were tested using 2-sample t test, 1-way analysis of variance with Tukey`s post-hoc test and with paired test. In study **IV,** paired samples t-tests were used to analyse changes in cognitive test performances from baseline to follow- up within each group (converters and non-converters). Independent samples t-tests were used to analyse differences in test performances between the converters and non-converters at baseline, as well as to analyse differences in cognitive decline from baseline to follow-up between the two groups. (Cognitive decline was quantified by calculating the difference between the test performances at baseline and at follow-up). Possible correlations between the regional

[^{11}C] PIB uptake at baseline and cognitive decline during the two year follow-up within each of the two groups (converters and non-converters) were analysed using Spearman Rank Correlation. All statistically significant correlations were examined by scatter plots in order to ensure that the correlation was not due to an outlier. A significance level of $p < 0.05$ was adopted.

5. RESULTS

5.1 Cerebrospinal fluid biomarkers in mild cognitive impairment (Study I)

In study **I,** the CSF levels of Aβ42, totalTAU and pTAU (^{181}P) were measured in MCI subjects. The cut-off values of biomarkers used were (in pg/ml): Aβ42<450, totalTAU>400, pTAU>70, Aβ42/pTAU<6.5. The means ± SD scores are presented in **Table 4.** In MCI subjects, Aβ42 was abnormal in 53%, totalTAU in 67%, pTAU in 64% and the Aβ42/pTAU ratio in 64% of subjects.

Table 4. The values of CSF biomarkers for MCI subjects; range, mean and standard deviation. n= number of MCI subjects, positive = number of CSF-positive subjects. % = percentage of all MCI subjects that have a positive CSF-marker. The table has been previously published in Original Publication **I.**

CSF	value range pg/ml	mean	SD	n	positive	%
Aβ42	275-731	469	143	15	8	53
totalTAU	171-959	507	243	15	10	67
pTAU	41-127	84	26	14[1]	9	64
Aβ42/pTAU	3.52-16,0[2]	6.43	3.46[2]	14[1]	9	64

[1]CSF pTAU was not available for one patient.

[2]Values refer to a ratio, and therefore are not in pg/ml.

5.2 [^{11}C] PIB uptake in mild cognitive impairment and in healthy controls (Study I)

[^{11}C] PIB uptake was increased in 87% of the MCI subjects in the posterior cingulate and in the neocortex. In the frontal cortex, the corresponding number was 80%. The values (mean ± SD) of the [^{11}C] PIB uptake ratio for MCI individuals and healthy controls are presented in **Table 5**.

Table 5. Results from automated ROI analyses of [11C]PIB uptake. Mean (SD) region-to-cerebellum ratio at 60-90 minutes in subjects with MCI and controls. p = p-value of two-sample t-test . The table has been previously published in Original Publication **I.**

Brain area	MCI	CONTROL	p
Anterior cingulate	1.74 (0.48)	1.08 (0.20)	<0.0001
Posterior cingulate	1.84 (0.41)	1.18 (0.18)	<0.0001
Lateral frontal cortex	1.51 (0.33)	1.01 (0.14)	<0.0001
Medial frontal cortex	1.74 (0.47)	1.05 (0.19)	<0.0001
Caudate	1.48 (0.32)	1.06 (0.15)	<0.0001
Putamen	1.72 (0.35)	1.27 (0.08)	<0.0001
Thalamus	1.52 (0.17)	1.35 (0.05)	<0.0001
Occipital cortex	1.33 (0.16)	1.12 (0.08)	<0.0001
Lateral temporal cortex	1.60 (0.32)	1.13 (0.13)	<0.0001
Parietal cortex	1.56 (0.29)	1.10 (0.15)	<0.0001
Medial temporal lobe	1.14 (0.12)	1.03 (0.08)	0.0027
Pons	1.61 (0.20)	1.54 (0.11)	0.1730
Neocortical score	1.57 (0.29)	1,11 (0.06)	<0.0001

ROC (receiver operating characteristic) analysis was performed to compare the [^{11}C] PIB uptake values in different brain areas between MCI subjects and controls. The ROC values indicate that the neocortical [^{11}C] PIB uptake score and [^{11}C] PIB uptake in the anterior cingulate most significantly could differentiate patients with MCI from controls. The area under the curve value (AUC) in the anterior (p=0.0014) and posterior (p=0.0007) cingulate was 0.93, in the lateral frontal cortex 0.94 (p=0.0011) and in the neocortex 0.96 (p=<0.0001). [^{11}C] PIB uptake was also analysed by calculating the distribution volume ratio (DVR) (Logan et al 1998), which yielded essentially similar results as obtained with the region-to-cerebellum ratio method. The correlation between ROI and DVR was r=0.52-0.81, p=0.002 to <0.0001.

5.3 The neocortical [^{11}C] PIB uptake and cerebrospinal fluid biomarkers (Study I)

When comparing the neocortical [^{11}C] PIB uptake scores and CSF biomarker values, only 54% of PIB-positive subjects showed AD-type (<450 pg/ml) Aβ42 values. The corresponding figures were 69% for totalTAU, 63% for pTAU and 67% for the Aβ42/pTAU ratio.There was only one subject who had an abnormal Aβvalue in the CSF, but in whom the uptake of [^{11}C] PIB was normal (**Figure 6a)**. In addition one subject had both normal CSF biomarkers and [^{11}C] PIB uptake ratio (**Figure 6a-c)**. There was no statistically significant correlation between Aβ42 levels and the [^{11}C] PIB uptake ratio in the lateral frontal cortex and posterior cingulate, or between the CSF Aβ42 levels and the neocortical [^{11}C] PIB score.

Figure 6a

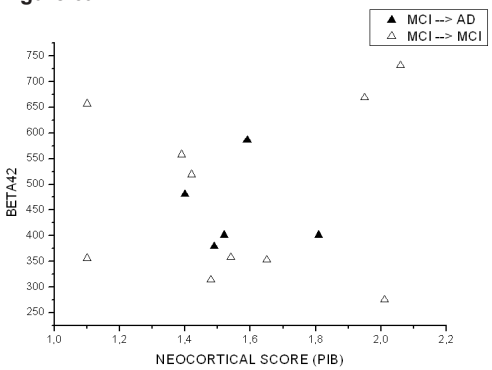
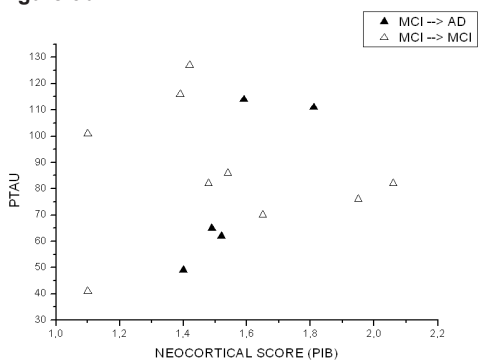


Figure 6b



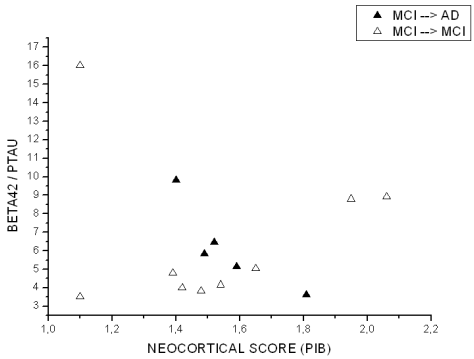


Figure 6c

Figure 6a-c. The association of the neocortical [^{11}C] PIB uptake score with CSF Aβ42(a), pTAU(b) and the Aβ42/TAU ratio(c) in MCI individuals.These figures have been reasently published in Original Publication **I.**

During a 2-year follow-up, 40% of the MCI subjects had converted to AD. All of these individuals had increased [^{11}C] PIB uptake ratios in the posterior cingulate and in the frontal cortex, or increased neocortical [^{11}C] PIB scores at the MCI stage. CSF Aβ42 and pTAU was abnormal in 20% of the subjects and the Aβ42/pTAU ratio in 26% of the subjects at the MCI stage. At the group level, there was no significant difference in [^{11}C] PIB uptake, CSF Aβ42, pTAU or the Aβ42/pTAU ratio between the MCI individuals who later converted to AD as compared to those who remained at the MCI stage.

5.4 [^{11}C] PIB uptake in mild cognitive impairment subgroup at baseline and the three year clinical follow-up: ROI analysis (Stydy II)

Baseline: When compared to controls as a goup, the subjects with MCI exhibited a 41% increase in PIB retention in the anterior cingulate ($p<0.01$), 40% increase in the posterior cingulate ($p<0.01$), 37% increase in the frontal cortex ($p<0.01$), 28% increase in the temporal cortex ($p<0.01$) and 20% increase in the occipital cortex ($p<0.01$). Altogether 55% subjects with MCI had significantly increased PIB uptake as compared to controls at baseline in all cortical ROIs. The age, gender, MMSE score and mean PIB retention ratio values in the subjects with MCI are presented in **Table 6.**

Table 6. Demographic data and mean [^{11}C] PIB retention ratio values in MCI subgroups. Data is presented as mean ± SD when appropriate.

	MCI	MCI converters	MCI nonconverters	controls	p Value
Number of subjects	31	15	16	26	
Age, y	69.4±7.9	71±6.3	67.9±9.0	65.3±6.5	NS
Male/female	19/12	9/6	10/6	11/15	
MMSE at baseline	27.5±1.5	27.1±1.5	27.9±1.3	29.5±0.8	NS
PIB retention ratio					
Anterior cingulate	1.61±0.49	1.98±0.37	1.26±0.28	1.13±0.08	<0.01☼
Posterior cingulate	1.68±0.54	2.10±0.41	1.30±0.32	1.19±0.13	<0.01☼
Frontal	1.50±0.43	1.82±0.36	1.20±0.23	1.09±0.06	<0.01☼
Temporal	1.40±0.38	1.66±0.36	1.16±0.18	1.09±0.06	<0.01☼
Parietal	1.51±0.44	1.82±0.38	1.21±0.24	1.10±0.07	<0.01☼
Occipital	1.37±0.33	1.59±0.35	1.17±0.11	1.14±0.07	<0.01☼

☼ Statistical significant difference in PIB retention ratio values between MCI converters compared to MCI nonconverters (univariate analysis of variance).
MCI = mild cognitive impairment; NS = no significant difference between MCI converters compared to MCI non-converters(Student t test, two-tailed); MMSE=Mini-Mental State Examination; PIB=Pittsburgh compound B.

Follow-up: During a 12-36 month follow-up, 48% of MCI subjects clinically converted to AD. From these, 82% had been PIB-positive also at baseline. When comparing the converters and nonconverters, the converters showed higher PIB retention in all cortical brain regions than the non-converters ($p<0.01$ both with and without correction for age) **Table 6.**

As a group, the PIB-positive faster converters had higher PIB retention values compared to the combined subgroup of slower converters and nonconverters in the anterior cingulate ($p=0.027$) and frontal cortex ($p=0.031$) **Table 7.** MCI faster converters might have already reached their amyloid load plateau, while the slower converters had lower levels of amyloid load and this might represent a prodromal or incipient AD phase at the time of scanning.

Table 7. Demographic data and [^{11}C] PIB mean retention ratio values in PIB- positive MCI subgroups

	PIB-positive faster converters (n=8)	PIB-positive slower converters (n=6) and nonconverters (n=3)	p Value	p Value☼
Total no. of patients	8	9	-	-
Age, y	71.5±7.1	70.6±5.3	NS	-
Male/female	4/4	6/3	-	-
MMSE at baseline	27.0±1.5	27.7±1.7	NS	-
PIB retention ratio				
Anterior cingulate	2.17±0.38	1.82±0.11	0.03	0.04
Posterior cingulate	2.25±0.42	1.98±0.21	0.13	1.15
Frontal	2.01±0.39	1.65±0.12	0.03	0.04
Temporal	1.83±0.40	1.51±0.17	0.07	0.09
Parietal	1.95±0.42	1.71±0.20	0.18	0.23
Occipital	1.67±0.45	1.47±0.18	0.26	0.33

Values are mean ± SD. p Value relates to PIB-positive MCI faster converters compared to PIB-positive MCI slower converters and nonconverters (univariate analysis of variance).
☼ Age as covariate. PIB = Pittsburgh compound B; MCI = mild cognitive impairment; NS = no significance between PIB-positive faster converters compared to PIB-positive slower and nonconverters (Student t test, two-tailed); MMSE = Mini-Mental State Examination.

5.5 APOE ε4 status (Study II and III)

An APOE ε4 allele was present in 41% of MCI subjects (Study **II**). Of these APOE ε4 carriers 86 % were PIB-positive. There was an association between the APOE ε4 status in the PIB-positive subjects with MCI and the rate of conversion (p=0.035). In contrast, only 33% of the PIB-positive slow converters were APOE ε4 carriers.

In study **III**, 82% of the converters were APOE ε4 positive. From these APOE ε4 positive subjects, 57% were homozygous for the ε4 allele. In the non-converters APOEε4 positivity was less frequent, with only 25% of the subjects having one ε4 allele. None of non-converters were homozygous for the ε4 allele. In the controls, 31% had one ApoE ε4 allele and none were homozygous.

5.6 [^{11}C] PIB uptake and hippocampal atrophy during follow-up (Study III)

[^{11}C] PIB uptake at baseline:

[^{11}C] PIB uptake was increased in converters and non-converters in the anterior cingulate (p=0.0004), in the posterior cingulate (p<0.0001), in the lateral frontal cortex (p<0.001), in the temporal cortex (p=0.0002), in the lateral parietal cortex (p=0.0006) and in the caudate nucleus (p=0.0004) as compared to healthy controls. **Table 8.**

Table 8. [11C]PIB uptake values (mean; SD) in converters, non-converters and in healthy controls during 24 month follow-up. [11C]PIB uptake is expressed as a region-to-cerebellum ratio at 60-90 min after injection. % = percentage of baseline value. The table has been previously published in Original Publication **III**.

	Converters			**Non-converters**			**Controls**
	Baseline	Follow-up	%	Baseline	Follow-up	%	
Anterior cingulate	1,71;0,23 b	1,76;0,21	103	1,51;0,32	1,58;0,32 †	105	1,39;0,11
Posterior cingulate	1,91;0,25 c,*	1,97;0,25	103	1,59;0,44 a	1,68;0,43 †	106	1,29;0,14
Frontal cortex	1,57;0,17 c,+	1,60;0,18	102	1,34;0,25	1,39:0,26	104	1,25;0,10
Temporal cortex	1,52;0,16 c,*	1,53;0,17	101	1,34;0,23	1,38;0,24 †	103	1,27;0,01
Parietal cortex	1,49;0,21 b	1,51;0,21	101	1,34;0,22	1,39;0,24 †	104	1,24;0,10
Putamen	1,67;0,23 b	1,73;0,25	103	1,48;0,25	1,55;0,26 †	105	1,41;0,13
Caudate nucleus	1,64;0,25 c,*	1,64;0,25	100	1,37;0,37	1,41;0,41	103	1,25;0,13

a = $p<0.05$, b = $p<0.01$, c = $p<0.001$ comparing converters and non-converters to controls at baseline.
* = $p<0.05$, + = $p<0.01$ comparing converters and non-converters at baseline.
† = $p<0.05$ comparing follow-up and baseline.

Converters showed a mean 62% increase in [11C] PIB retention in the posterior cingulate, 32% increase in the anterior cingulate, 32% increase in the lateral frontal cortex, 39% increase in the caudate nucleus, 26% increase in the putamen, 25 % increase in the temporal cortex and 25% in the lateral parietal cortex compared to healthy controls. Non-converters showed in the same brain areas 30% (posterior cingulate), 12% (anterior cingulate), 12% (caudate nucleus), 10% (lateral parietal cingulate), 9% (lateral frontal cortex), 7% (temporal cortex) and 7% in the putamen (see **Table 8**). The results of the voxel-wise statistical analyses were in agreement with the results of regional analyses showing significant group differences in [11C] PIB uptake in many brain areas. At baseline, [11C] PIB uptake was greater in converters compared to the non-converters in the anterior cingulate (p=0.068), the posterior cingulate (p=0.020), the lateral frontal cortex (p=0.004), the temporal cortex (p=0.019), the putamen (p=0.052) and in the caudate nucleus (p=0.025). Individually, increased , [11C] PIB uptake values (defined as region-to-cerebellum ratio equal or greater than 1.5) were noted more frequently in the converters than in the non-converters; anterior cingulate 82% vs 42%, posterior cingulate 94% vs 42%, lateral frontal cortex 65% vs 33%, parietal cortex 41% vs 33% and temporal cortex 53% vs 33%. It was also notable, that 2 of healthy controls had increased [11C] PIB uptake in the anterior and posterior cingulate.

Hippocampal atrophy at baseline:

The converters had greater atrophy scores than the controls in the left (p=0.023) hippocampus. In the non-converters, the mean atrophy score was greater in the right hippocampus (p=0.023) as compared to controls. The mean hippocampal atrophy scores in the converters and non-converters are presented in **Table 9**.

Table 9. The hippocampal atrophy scores (mean; SD) of the MCI converters, non-converters and controls. Mean; SD. The table has been previously published in Original Publication **III**.

	Converters		Non-converters		Controls
	Baseline	Follow-up	Baseline	Follow-up	Baseline
Left	1,94; 1,14 [a]	2,41; 1,06 [b]	1,42; 1,08	2,00; 1,48 [c]	0,85; 0,69
Right	1,65; 1,00	2,18; 1,01 [b]	1,75; 0,76 [a]	2,08; 1,00 [b]	0,85; 0,69

a = p <0.05 as compared to controls; b = p<0.0001, c = p <0.01 as compared to baseline

[^{11}C] PIB uptake at follow-up:

During the two year follow-up, 59% of MCI subjects had clinically converted to AD. The mean [^{11}C] PIB uptake values in both converters and non-converters are presented in **Table 8.** In the converters, [^{11}C] PIB uptake values ranged only from 100% to 103% of the baseline value at the follow-up. When examining the non-converters, [^{11}C] PIB uptake was increased in the anterior cingulate; mean 5% (p=0.028), posterior cingulate; 6% (p=0.011), temporal cortex; 3% (p=0.041), parietal cortex; 4% (p=0.014), and putamen; 5% (p=0.028). The individual [^{11}C] PIB uptake values in the posterior cingulate are presented in **Figure 8.** The difference in the change in [^{11}C] PIB uptake between baseline and follow-up was not significantly different between converters and non-converters. Voxel-wise analyses were in agreement with the ROI based findings. Higher [^{11}C] PIB uptake at follow-up compared to baseline was seen in non-converters, especially in frontal lobe and posterior cingulate.

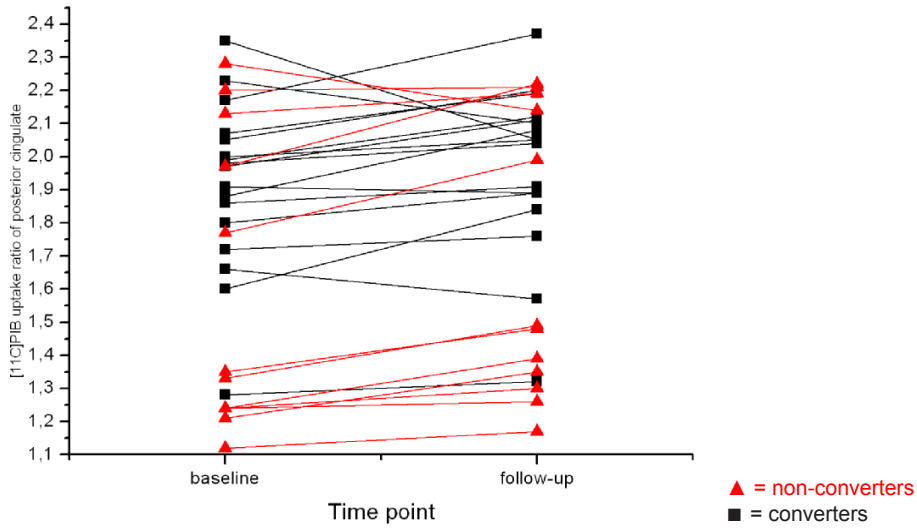


Figure 8. Individual [^{11}C] PIB Pittsburgh compound B (PIB) uptake values at baseline and follow-up in converters and non-converters in the posterior cingulate. The figure has been previously published in Original Publication **III**.

Hippocampal atrophy at follow-up:

Hippocampal atrophy increased during the follow-up both in the converters ($p<0.001$ for both right and left hippocampus) and in the non-converters ($p<0.001$ for right and $p=0.004$ for left hippocampus). The mean hippocampal atrophy values are presented in **Table 9**.

5.7 MMSE (Study III and IV)

The mean MMSE score of the MCI subjects in study **III** was 26.9; SD 1.6 at baseline. The corresponding value in the healthy controls was 28.2; SD 1.5 ($p=0.018$). When divided into the converters and non-converters; the mean score of the converters was 26.2; SD 1.3 and non-converters 27.9; SD 1.3 ($p=0.004$). During the two year follow-up, the mean MMSE score decreased by 3.82 points in the converters but only by 1.08 in non-converters ($p=0.001$; difference in the change between the groups). At follow-up, the MMSE score of the converters was 22.4; SD 2.7 compared to 26.8; SD 1.1 for non-converters. The mean MMSE score of the 16 MCI patients in study **IV** was 26.8 (SD 1.5) at baseline. There was a statistically significant difference ($p<0.01$) between the converters (mean; SD 26.3, 1.6) and the non-converters (mean;SD 27.8, 1.2) in baseline MMSE-scores. At follow-up, the mean MMSE scores were 22.3 (SD 2.6) in the converters and 26.7 (SD 1.0) in the non-converters. The mean MMSE score decreased in the converters by 4.0 points and in the non-converters by 1.1 points and thus the difference in MMSE-decline between the groups was statistically significant ($p<0.01$).

5.8 Psychometric test scores and [^{11}C] PIB uptake in mild cognitive impairment (Study IV)

Psychometric test scores at baseline and at follow-up are presented in **Table 10.** At baseline, there were no statistically significant differences in test scores (except for the MMSE) between the converters and non-converters. In the MCI individuals who later converted to AD, statistically significant decline from baseline to follow-up was observed in word list learning, word list delayed recall, word list savings, clock drawing, trail A time and trail AB difference ($p < 0.05$). In the MCI subjects who remained as MCI at follow-up, a statistically significant decline was only observed in the MMSE. In the extent of the decline from baseline to follow-up (*i.e*. the difference in test scores between the two measurement points), there was a statistically significant difference in word list delayed recall and word list savings between the two groups ($p < 0.05$). In these two test scores, there was a relatively marked decline in the converters, while no changes were observed in the non-converters.

Table 10. Psychometric test scores at baseline and at follow-up in the converters and non-converters subgroups (mean, SD), as well as the statistical significance of within-groups change (baseline vs. follow-up) and between-groups differences in A) test performances at baseline and B) the degree of test performance decline during follow-up (* = statistical significance p <0.05, NS = non-significant).

	Converters (n=9)			Non-converters (n=7)				
	Baseline (BL)	Follow-up (FU)	Sign. (BL –FU)	Baseline	Follow-up	Sign. (BL –FU)	A	B
MMSE	26.3 (1.6)	22.3 (2.6)	*	27.8 (1.2)	26.7 (1.0)	NS	*	*
Word fluency	19.7 (6.1)	15.7 (6.1)	NS	20.6 (6.6)	20.7 (5.2)	NS	NS	NS
Naming	13.7 (1.2)	12.6 (2.0)	NS	13.0 (2.1)	12.7 (1.9)	NS	NS	NS
Word list learning	19.0 (4.4)	15.3 (5.0)	*	21.4 (6.0)	21.6 (2.1)	NS	NS	NS
Word list del. recall	5.6 (2.1)	3.0 (2.8)	*	6.9 (2.2)	6.7 (2.0)	NS	NS	*
Word list savings	74.0 (19.2)	36.9 (35.7)	*	86.0 (10.1)	85.7 (24.1)	NS	NS	*
Clock drawing	5.6 (0.7)	3.6 (1.9)	*	5.6 (0.9)	4.9 (1.5)	NS	NS	NS
Stroop interference	82.1 (30.4)	107.0 (82.8)	NS	52.0 (27.0)	91.7 (65.1)	NS	NS	NS
Trail A time	73.3 (24.9)	61.0 (20.4)	*	89.7 (41.0)	151.3 (143.7)	NS	NS	NS
Trail AB difference	75.13 (50.2)	125.4 (125.8)	*	72.6 (53.4)	168.3 (173.0)	NS	NS	NS

[^{11}C] PIB uptake at baseline

At baseline, the converters (C) had significantly higher [^{11}C] PIB uptake in several brain areas compared to the non-converters (NC); statistically significant differences ($p < 0.05$) were observed in the anterior cingulate (C = 1.69; NC = 1.33), the posterior cingulate (C = 1.91; NC = 1.32), the lateral frontal cortex (C = 1.55; NC = 1.20), the medial frontal cortex (C = 1.67; NC = 1.23), the temporal cortex (C = 1.50; NC = 1.20), the parietal cortex (C = 1.46; NC = 1.24), the putamen (C = 1.65; NC = 1.33) and the caudate nucleus (C = 1.62; NC = 1.17).

[^{11}C] PIB uptake and psychometric test score decline

In the converters, there were several statistically significant correlations between baseline [^{11}C] PIB uptake and the decline in the psychometric test score. Lower baseline [^{11}C] PIB uptake in frontal areas was associated with greater decline in word list learning ($r = -0.73$, $p < 0.05$). The decline in trail making test A was associated with higher baseline [^{11}C] PIB uptake in the caudate nucleus ($r = 0.78$, $p < 0.05$), and the putamen ($r = 0.90$, $p < 0.01$). The higher baseline uptake in the caudatus was also associated with a faster decline on word list savings ($r = 0.73$, $p < 0.05$). Higher baseline [^{11}C] PIB uptake in the temporal cortex was associated with greater decline in the Stroop test ($r = 1.00$, $p < 0.01$).

In the non-converters, the decline in the clock drawing test was associated with higher baseline [^{11}C] PIB uptake in the anterior cingulate ($r = 0.95$, $p < 0.05$), and the medial frontal cortex ($r = 0.95$, $p<0.05$). Higher [^{11}C] PIB baseline uptake in the putamen was associated with a faster decline in word-list delayed recall ($r = 0.93$, $p < 0.05$).

6. DISCUSSION

MCI represents a transitional state between normal aging and different dementia disorders. In particular aMCI might represent an early AD state. However, some aMCI individuals will remain stable for many years, some even recover. One major future challenge will be to detect at an early stage those MCI subjects, who are in risk to convert to AD or other dementias. In the future new AD mechanism-based therapies; Aβ42 immunotherapy, γ-secretase inhibitors and β-sheet breakers; might prove to have disease-arresting or decelerating effect. These drugs are likely to have their best efficacy during the presymptomatic state of the disease, when the synaptic and neuronal damage has not become too widespread. Thus, methods to predict presymptomatic AD individuals are expected to be of considerable importance.

6.1 [^{11}C] PIB PET in mild cognitive impairment and Alzheimer`s Disease (Studies II and III)

Aβ-specific PET radiotracers allow quantitative analysis of Aβ accumulation *in vivo.* This is important not only for the diagnosis of AD, but also in antiamyloid drug development and research. The best validated of these radiotracers is N-methyl-2-(4`-methylaminophenyl)-6-hydroxybenzothiazole, *i.e.* 6-OH-BTA-1; `Pittsburgh Compound-B`([^{11}C] PIB), a carbon-11- labelled derivative of the thioflavin-T amyloid due. [^{11}C] PIB binds with very high affinity and high specificity to neuritic Aβ-plaques (Klunk et al. 2004, Klunk et al. 2003). [^{11}C] PIB uptake is evidently higher in frontal, parietal, lateral temporal and posterior cingulate / precuneus brain cortices when compared healthy elderly individuals and AD patients (Scheinin et al. 2009, Rowe et al. 2007, Kemppainen et al. 2006, Klunk et al. 2004). Similar findings have been reported also in MCI subjects, and increased [^{11}C] PIB uptake in MCI has been reported to predict conversion to AD (Forsberg et al. 2008, Pike et al. 2007).

In studies **II** and **III**, we found that subjects with MCI had increased [^{11}C] PIB uptake in frontal, parietal, lateral temporal and occipital cortices, anterior and posterior cingulate, caudate and putamen as compared to healthy controls. The uptake was significantly higher at baseline in those MCI individuals who subsequently converted to AD during follow-up. In study **II,** almost half of the MCI subjects converted to AD during one year and as many as 82% during the 3 year follow-up. In study **III** the conversion rate was 59% during two years. The higher conversion rates to AD in the PIB-positive compared to PIB-negative subjects with MCI in these two studies indicate that *in vivo* detection of amyloid deposition may provide useful prognostic information with respect to stratifying those MCI individuals at increased risk of AD. A recently published one-year follow-up study in MCI subjects is in agreement with the present results, *i.e.* “PIB-positive” MCI subjects have higher conversion rate to AD than “PIB-negative” patients (Jack et al. 2009).

In a longitudinal [^{11}C] PIB PET two year follow-up study of patients with early AD (Engler et al. 2006), it was found, that the amyloid load in the AD group remained relative stable. In study **III** in the MCI individuals, the converters did not exhibit a significant increase in [^{11}C] PIB uptake during follow-up whereas the non-converters displayed increased uptake in frontal, parietal, lateral temporal cortices, as well as in anterior and posterior cingulate and putamen. This finding suggests that amyloid deposition occurs early in the clinical evolution of AD and [^{11}C] PIB retention will change only modestly when conversion to AD is occurring. It was found also in study **II** that those MCI subjects who converted faster, during a one year to AD, had a significantly increased amyloid burden in the anterior cingulate and frontal cortices compared to the slower converters and non-converters. Together these findings in studies **II** and **III** are evidence that converters (faster converters) may have already reached their amyloid load plateau. This would fit with the theoretical proposals of a sigmoidal type of increase in Aβ load in the transition from normal ageing to MCI and AD, Figure **2** (2.2).

However, a longer follow-up time would be needed to determine if converters would undergo a slow increase in [^{11}C] PIB uptake and whether some of the non-converters would convert later to AD. The subject groups here were quite small and there was no significant difference in the change in [^{11}C] PIB uptake from baseline to follow-up between converters and non-converters, and thus in the future it would be important to study larger number of MCI individual.

Increased amyloid deposition is a characteristic feature of subjects with MCI and AD. However, one must bear in mind that amyloid is also present in many normal older people, as many as a third of healthy older adults might appear to significant deposition (Rodrique, Kennedy and Park 2009). It is not known why some individuals with increased amyloid deposition experience clinical symptoms and some stay cognitively normal in spite of the same degree of amyloid accumulation. There are several possible explanations not all of which are limited to different cognitive reserve, *e.g.* APOE or other genetic factors, environmental factors or their combinations may be involved. The role of the Aβ-protein in the brain is not entirely clear, but it has been assumed that the soluble form of Aβ may cause synaptic dysfunction (Nordberg 2008, Selkoe 2002). The amount of extracellular soluble Aβ in the brain is probably a better predictor of cognitive impairment in AD than the number of plaques themselves (Nordberg 2008). In the future it would be extremely important to understand the precise neural mechanism that determine cognitive outcomes in late adulthood as well as identifying markers of less successful cognitive aging as early as possible.

6.2 Hippocampal atrophy in mild cognitive impairment and Alzheimer`s Disease (Study III)

In AD research, MRI is used in the assessment of brain atrophy and evaluation in the evaluation of hippocampus, entorhinal and temporal neocortical volumes. Structural

MRI captures disease-related structural changes in the brain by measuring loss of brain volume which is a direct result of loss of neurons, synapses, and supporting cellular structures. Generally hippocampal atrophy is present in both MCI and AD patients compared to healthy individuals and seems to be increased during AD pathology. MRI scan has a significant value in being able to discriminate MCI individuals at risk of developing AD (Vemuri et al. 2009, Chetelat and Baron 2003).

In study **III**, hippocampal atrophy was visually evaluated in MRI images being scored from 0 (no atrophy) to 4 (severe atrophy) (Scheltens et al. 1992) by an experienced neuroradiologist who was blinded to the clinical status of the subjects. At baseline, when compared to control subjects hippocampal atrophy was present in both MCI groups, converters and non-converters. In addition the atrophy increased in both groups during follow-up. This result is in agreement with the recently reported one year follow-up study in 32 MCI patients who demonstrated a clear increase in hippocampal atrophy during follow-up (Jack et al. 2008). This present study, it was found that hippocampal atrophy increased during follow-up in both converters and non-converters whereas [^{11}C] PIB uptake increased only in the non-converters. One explanation for this finding might be that [^{11}C] PIB uptake increases early and changes relatively little during the AD pathology whereas the atrophy increases progressively with time.

6.3 CSF biomarkers in mild cognitive impairment and Alzheimer`s Disease (Study I)

A few CSF proteins have shown promise as diagnostic biomarkers for clinical AD and even presymptomatic AD. Lower mean levels of CSF Aβ42 and higher mean levels of tau and phosphorylated tau have been reported to be able to distinguish groups with clinical AD from cognitively normal controls (Hansson et al. 2006, Andreasen and Blennow 2005). Clinicopathological studies have also shown that low ventricular CSF levels of Aβ42 correlate inversely with the amyloid load in the brain, suggesting that the CSF concentration of Aβ42 does reflect brain pathology (Strozyk et al. 2003). A definitive diagnosis of AD depends on finding neuritic plaques and neurofibrillary tangles in the brain in a context of progressive cognitive decline. Together with MRI and PET imaging techniques and a clinical examination, low Aβ42 concentrations combined with high tau levels in CSF, have been suggested as being biomarkers of value in early clinical diagnosis (Sjogren et al. 2001). Several studies have shown that individuals with MCI who progressed to AD had an increased baseline concentration of totalTAU and pTAU^{181} , whereas the concentration of Aβ42 was decreased (Hansson et al 2006, Herukka et al. 2005, Hampel et al. 2004).

In MCI , the profiles of AD-type CSF biomarkers predict rapid progression and conversion to AD. In addition, it is known that [^{11}C] PIB uptake is increased in MCI and AD. It was interesting to examine in study **I** the relationship between these biomarkers, since this has not been studied extensively in MCI. In this study it was found that subjects with aMCI

have increased [^{11}C] PIB uptakes in the lateral frontal cortex and posterior cingulate, and at the same time, increased CSF pTAU levels and a decreased Aβ42/pTAU ratio. In a population-based autopsy study, there was a strong inverse association between post-mortem ventricular CSF Aβ42 levels and the number of neuritic plaques (Strozyk et al. 2003). Even in non-demented individuals, a decrease in the Aβ42 level reflected an increasing plaque load. Low Aβ42 levels may reflect the neuropathological processes implicated in amyloid-related pathologies, such as neuritic plaques. It is possible that clearance of Aβ42 from the brain into CSF decreases as this peptide accumulates in the plaques. Here, it seemed that there was only a weak correlation between CSF Aβ42 concentrations and [^{11}C] PIB uptake. One possible reason for the discrepancy is that [^{11}C] PIB uptake reflects fibrillar (plaque) amyloid and CSF Aβ42 reflects amyloid excreted from the brain into CSF. However in other studies in MCI and AD, a negative association has been found between CSF Aβ42 and cerebral [^{11}C] PIB uptake (Forsberg et al. 2008, Fagan et al. 2006). A recent clinicopathological case study indicated that [^{11}C] PIB binding does not distinguish between the amyloid in plaques and in blood vessels (Bacskai et al. 2007). It might be possible that the weak correlation in this study might reflect the different micromolecular structures of Aβ42 in fibrillar plaques and in CSF. It is well known that the soluble form of Aβ-protein seems to cause the dysfunction within the synapses. However, it is still not known why some healthy individuals with amyloid accumulation in the same cortical areas as observed in MCI and AD patients, are cognitively healthy. The weak correlation in this present study may also reflect very dynamic CSF Aβ42 metabolism, since the hour-to-hour variation within individuals is considerable. This biological variation may influence the value of CSF Aβ42 (Bateman et al. 2007). There were also certain weaknesses in this study. The patient group was quite small; 15 aMCI and 22 healthy controls; and the time lapse (up to 12 months) was relatively long between CSF sampling and PET examination. Thus longer follow-up studies will be needed to evaluate the relative predictive value of [^{11}C] PIB PET and CSF biomarkers for conversion from MCI to AD.

6.4 APOE ε4 in mild cognitive impairment and Alzheimer`s Disease (Study II and III)

In addition to the three parameters described above *i.e.* hippocampal atrophy in MRI, AD-type CSF profile, increased [^{11}C] PIB uptake, APOE ε4 has also been proposed as a predictor of conversion to AD (Hampel et al. 2004, Blennow and Hampel 2003, Petersen et al. 2000, Jack et al. 1999). Many studies have also demonstrated an association between APOE ε4 allele and decreased levels of Aβ42 in CSF in patients with AD (Prince et al. 2004, Sunderland et al. 2004), and in cognitive healthy subjects at high risk for dementia due a family history of AD (Sunderland et al. 2004). In addition increased hippocampal atrophy has been shown to be in association with APOE ε4 allele status in MCI and AD patients (Jack et al. 2008). In one study it was found that levels of Aβ protein deposition varied according to APOE genotype in elderly subjects both with and without dementia,

with the highest mean values being associated with the presence of at least one APOE allele (Polvikoski et al.1995).

In study **II,** the APOE ε4 allele was present in 41% of the subjects with MCI; 7 out of 17 patients. Six of these 7 APOE ε4 carries (86%) were PIB-positive. The APOE ε4 allele was not known in 14 MCI participants. There was a significant association between APOE ε4 status and increased [^{11}C] PIB uptake; and rate of conversion to AD. All four faster converters were APOE ε4 positive, whereas only 2 out of 6 slow converters carried an ε4 allele. In one previous [^{11}C] PIB PET study in AD, it was found that APOE ε4 status might represent a possible confounding factor between clinical severity of dementia and amyloid plaque load (Grimmer et al. 2008). According to this study, the patients with more severe dementia had higher [^{11}C] PIB bilaterally in the frontal and anterior cingulate cortices and putamen. One limitation of the present study was that APOE ε4 status was not evaluated in all MCI participants. However, according to the present study findings, APOE ε4 status is a risk factor for AD.

In study **III,** 82% of those MCI subjects, who converted to AD during 2 year, were APOE ε4 positive and 30 % were homozygous. In contrast, in the non-converters, APOE ε4 positivity was less frequent, with only 25 % of the individuals having one ε4 allele and none of them were homozygous. In the controls, 31% had one ε4 allele and none were homozygous. Thus both studies **II** and **III** indicate that APOE ε4 increases the likehood of conversion from MCI to AD. A recent study also showed that even in cognitively normal elderly subjects, there is an increase in [^{11}C] PIB uptake according to ε4 allele load; no, one or two alleles (Reiman et al. 2009). In the present study, the combination of increased [^{11}C] PIB uptake and the presence of ε4 allele may have contributed to the conversion from MCI to AD. In the future larger MCI individual population will be needed to evaluate the value of the individual contributions of increased [^{11}C] PIB uptake and the presence of APOE ε4 allele to conversion from MCI to AD.

6.5 Cognitive functions and PIB uptake in mild cognitive impairment (Study IV)

Only cross-sectional studies have been conducted on the relationship between PIB-binding and cognitive functions. The results have revealed a relatively modest correlation between memory and other cognitive functions in healthy controls and MCI individuals, while no such relationship has been detected in AD patients (Jack et al. 2008, Pike at al. 2007). The relationship between PIB- binding was investigated in different brain areas and cognitive decline during two-year clinical follow-up in MCI subjects. The data showed that in those MCI individuals who converted to AD, higher baseline [^{11}C] PIB uptakes in the caudate nucleus and the putamen were associated with a greater decline on a test of executive function and psychomotor speed. Higher baseline caudate [^{11}C] PIB uptake also predicted a faster decline on a verbal memory test. Moreover, higher [^{11}C] PIB uptake in the temporal cortex was associated with a more extensive

decline in a test measuring executive function and inhibition. There are two possible ways to interpret these findings. One is that amyloid accumulation in specific brain areas predict the decline in specific cognitive domains. Based on previous studies on the neuroanatomical and neurophysiological bases for different cognitive functions, the relationship between executive decline and basal ganglia involvement is not an expected (extensively studied in Parkinson`s disease patients, *e.g.* Zgaljardic et al. 2006). The caudate nucleus has also been implicated as a brain structure associated with memory processes (White 2009). The association between inhibitory attention (Stroop) and temporal lobe structures is, however, not easy to corroborate with previous studies. Another possibility is the concept that increased amyloid accumulation in these analysed brain areas simply predicts conversion to AD and a more general cognitive decline. In fact, this second explanation may, in fact, find some support in the other main finding in this study, namely the negative correlation between frontal baseline PIB uptake and the decline in verbal memory in the converters. At first the fact that a lower baseline [^{11}C] PIB uptake in frontal areas was associated with a greater decline in verbal memory may seem somewhat unexpected. However, this finding may be related to the temporal and anatomical sequence of amyloid accumulation in AD, as the build-up typically starts mainly in frontal areas and then spreads to other cortical brain areas with basal ganglia and hippocampus being affected in the later course of the disease (Thal et al. 2002). It is thus possible that the frontal amyloid load had already reached its maximum in the converters at the baseline measurement. This would then probably also explain the fact that higher amyloid accumulation in the basal ganglia and temporal areas was involved in the decline of memory and executive functions.

One interesting finding was the fact that there were associations in both the converters and non-converters between baseline basal ganglia uptake and the decline in verbal memory decline. This finding may first of all reflect the involvement of basal ganglia in episodic memory processing per se (White 2009), but it may also indicate that at least some of the non-converters may, in fact, represent a very early prodromal stage of AD. A longer follow-up would be needed to determine whether some (and which) of the non-converters would convert later to AD.

There are some limitations in study **IV** . The subject groups in the current study were small, and single observation may influence greatly coefficients correlation in such small samples. However, care was taken in interpreting the correlations by checking for outliers. Nonetheless, larger sample sizes will be needed to confirm these preliminary results. It is also possible that some of the non-converters may be in an early prodromal stage of AD, and a subsequent follow-up study of these subjects could clarify the sequence of amyloid accumulation and cognitive decline in AD. In addition, the possible relationship between amyloid accumulation in certain brain areas and deficits in specific cognitive domains could ideally be studied retrospectively in a larger group of MCI individuals (both converters and non-converters), focusing on the associations between these factors both at baseline and at follow-up.

The results of this study however suggest that frontal amyloid uptake reaches its peak relatively early during the prodromal AD stages, and that the more general cognitive decline is predicted by increased PIB uptake in the basal ganglia and the temporal lobe. Further studies could elucidate whether relative PIB uptake ratios (frontal to striatal/ temporal binding) could be used to predict conversion from MCI to AD.

6.6 General discussion

The term MCI was introduced to depict individuals who have cognitive impairment (especially memory problems) but who do not fullfill the clinical criteria for dementia (Petersen et al. 1999). MCI was initially considered as a stage between normal ageing and AD. However, later it became clear that MCI, as such, is a heterogenous disorder: some individuals with MCI will progress to dementia, some will remain MCI and some will even revert to normal. Furthermore, when progressing, MCI may lead to different dementing diseases such as Alzheimer´s disease, dementia with Lewy bodies, frontotemporal dementia or to dementia related to Parkinson´s disease (Petersen 2004). The type of MCI (*e.g.* amnestic versus non-amnestic or single vs. multiple domain) has been postulated to predict to which dementing disease MCI might evolve. In particular subjects with amnestic MCI has often been shown to progress to AD, although contradictory results have also been published (Jack et al. 2008). Therefore, recently the usefulness of the concept of MCI has been questioned. Since the knowledge of typical clinical symptoms and their evolution in different dementing diseases has increased and for instance new biomarkers (such as CSF measurements or brain imaging) may show the typical pathological changes related to AD, terms such as presymptomatic or prodromal AD have been suggested. In addition the putative new clinical research criteria for AD (Dubois et al. 2007) emphasize the importance of progressing memory impairment accompanied by abnormal biomarker finding(s).

The US Alzheimer`s Association and the National Institute of Aging of the National Institutes of Health has recently published new criteria and guidelines for AD (april 19, 2011; published in the Lancet april 30, 2011). According to these criterias and guidelines, AD is postulated in three stages with a continuum between and within each stage. The first stage is a preclinical phase, which might last a decade or more. The second stage is characterised by mild cognitive impairment due to AD and the third stage is AD. In the first stage there is evidence of low cerebrospinal Aβ42 and increased amyloid tracer retention on PET and at the later stage there is evidence of neuronal degeneration or injury. There is currently no value in making a clinical diagnosis of AD on this stage because people might not progress to dementia in their lifetime. In the second stage cerebrospinal fluid biomarkers and imaging technology might help to increase the probability of the underlying diagnosis of AD. Still the main diagnosis is made clinically and by exclusion of other causes. The third stage is AD.

The MCI individuals in this thesis fulfilled the suggested clinical criteria for amnestic MCI. Many of the subjects turned out to have either an AD-type CSF profile or a positive PIB PET scan. In the light of current knowledge, these individuals could have also been designated as patients with prodromal AD. This is supported by the very high conversion of these individuals to AD even during this relatively short clinical follow-up.

The general limitations of the study include the relatively small number of patients in some experiments, which is often the case in studies involving several investigations such as PET- and MRI-scans, CSF-sampling and neuropsychological investigations. During follow-up, some patients are lost which may at least theoretically cause a selection bias since it is possible that only a certain type of patient remains in the study. However, in this study there was no difference in the baseline demographic, cognitive or imaging characteristics of those MCI subjects who remained in the study as compared to the ones that were lost to follow-up.

[^{11}C] PIB was used as a PET tracer. This is a well characterized ligand that binds to fibrillar plaque and vascular amyloid. However, other forms of amyloid such as soluble oligomers might be more important in the pathophysiology of AD than fibrillar (plaque) amyloid. However, there are currently no in vivo PET tracers that would selectively or preferentially bind to these oligomers. Moreover, the amyloid pool in the brain is believed to be in a dynamic equilibrium, so that removal of soluble amyloid forms may result in the release of beta-amyloid from plaques leading to degradation of amyloid plaques (Hyman et al. 1997). Thus PIB and other tracers, even though binding to fibrillar amyloid, could reflect also changes in other forms of amyloid. In the future, new tracers that would bind selectively to soluble forms of amyloid or to some other relevant pathologies seen in dementing disease (like hyperphosphorylated tau protein or alpha-synuclein) may help in identifying specific pathologies at a very early stage which may be increasingly important when disease-modifying therapies become available.

At the present the preclinical state of AD is proposed only as a framework for scientific research to better understand progression and predictors of progression and also early intervention with disease-modifying therapy. If Alzheimers`s dementia could be prevented by modifying biological risk factors in a similar way to modifyinf *e.g* lipid levels with statins, this thesis might mark an importat turning point in Alzheimer`s research.

7. CONCLUSIONS

[^{11}C] PIB uptake appears to be a more sensitive than CSF Aβ42 concentration in detecting an increased amyloid burden in MCI. In MCI individuals, CSF Aβ42 levels were abnormal in 53% of subjects in study **I.** A composite neocortical [^{11}C] PIB uptake score was elevated in 87% of the MCI individuals. In other words, even though MCI subjects may have a normal CSF Aβ42 level, [^{11}C] PIB uptake might be increased.

MCI subjects with increased [^{11}C] PIB uptake are significantly more likely to convert AD than MCI subjects with negative [^{11}C] PIB uptake. The MCI subjects, who convert to AD during a brief time, so called "faster converters", might have higher [^{11}C] PIB retention levels at baseline than "slower converters". The detection of amyloid deposition in MCI individuals with [^{11}C] PIB PET might provide useful *in vivo* prognostinc information in future.

Hippocampal atrophy and amyloid deposition seem to dissociate during the evolution of MCI. The atrophy seems to increase clearly whereas [^{11}C] PIB retention changes more modestly when conversion to AD occurs. Those MCI subjects converting to AD might have greater [^{11}C] PIB retention in the posterior cingulate, the lateral frontal cortex, the temporal cortex, the putamen and in the caudate nucleus as compared to non-converters. In converters [^{11} C] PIB uptake appears to be quite stable during the follow-up, whereas it increases as compared to baseline in non-converters in the anterior and posterior cingulate, temporal and parietal cortices and in the putamen. Hippocampal atrophy seems to increase significantly in both converters and non-converters during follow-up.

Higher PIB uptake in the nucleus caudatus and the temporal lobes may predict conversion to AD. These results also indicate that in prodromal AD, frontal amyloid accumulation reaches its maximum in the clinical MCI stage that is characterized by isolated memory problems without the full-blown dementia syndrome.

8. ACKNOWLEDGEMENTS

This work was carried out at Turku PET Centre during the years 2006-2011. I wish to express my appreciation to Juhani Knuuti, Director of the Turku PET Centre, for allowing me to use the PET facilities, and for creating such a scientific atmosphere in which to work. I wish to express my gratitude to my daily co-workers, the core personnel of Turku PET Centre: Tuula Tolvanen and Mika Teräs for their expertise in matters of PET physics and instrumentation; Vesa Oikonen for his expertise in modelling; Rami Mikkola and Marko Tättäläinen for computer assistance. I wish to express special thanks to Tarja Keskitalo, Marjo Tähti, Minna Aatsinki, Hannele Lehtinen, Kalevi Mölsä, Anne-Mari Jokinen, Lehtimäki Leena, Heidi Betlehem, Sanna Suominen, Eija Nirhamo, Heidi Lappalainen and Emilia Puhakka for their excellent technical assistance. For secretarial matters, Mirja Jyrkinen and Sinikka Lehtola are gratefully acknowledged.

These studies were carried out with the help of several experts, without whom this work would not have been possible. I wish to thank Sargo Aalto for his help in [^{11}C]-PIB quantitation in these studies. I am grateful to Nina Kemppainen for her enormous help in orientation to research work and helping me with these studies. Jere Virta is acknowledged for help in methodology in study **III**. I wish to thank Tero Vahlberg, Vesa Mäkiaho and Mari Koivisto for their assistance in statistical matters. The personel of the radiochemistry laboratory of the University of Turku is thanked for the production and delivery of the radiotracer. I wish to thank Kjell Någren for his constructive comments about studies **I** and **III.** Matti Viitanen is warmly acknowledged for his valuable support and advice and also he had a role in finding some of the patients in these studies**.** I am grateful to Riitta Parkkola for her expertise with MRI images in study **III.** I express my gratitude to the late Tuula Pirttiä from University of Kuopio, for her constructive teamwork and her role in finding patients in study **I.** and for her valuable comments about study **I.** I wish also to thank Mira Karrasch for her professional help in analyzing the neuropsychological test results in study **IV.** I express my gratitude to her vast knowledge and integrated visions of neuropsychology science, her professional help was essential in completing study **IV.**

Esko Vanninen and Anne Remes, the official reviewers of this thesis, are warmly acknowledged for their professional advices. I am grateful also to Ewen MacDonald for revising the language.

My deepest feelings of gratitude and respect are addressed to my supervisors, Juha O. Rinne. Without his invaluable help and support this work would not have been possible. His never-ending encouragement, optimistic attitude and professional help have been essential in completing this thesis. I also want to express my gratitude to him for introducing me to the interesting world of science and the basics of brain research.

I wish to thank my fellow researchers; Terhi Tuokkola, Noora Scheinin, Anna Bruck, Jere Virta, Pekka Jokinen, Timo Suotunen, Eero Rissanen, Jyri Virta and Juho Joutsa for sharing this experience. I am gratitude for their help with the methodology and for sharing the scanning workload and for creating such a great atmosphere. Especially Noora is acknowledged for her support and advice.

I wish to thank all my dear friends, especially Tarja, Nella and Mika, Heli, Laura, Kirsi, Elisa, Anna, Johanna, Eve and Tommi, Pera, Erja, Aleksi and Saara for providing me with other perspectives outside science work and sharing the ups and downs of life.

To my parents, Airi and Jouko Matoniemi, as well as my sister, Tuija and her husband Kari and the family and my brother Jussi and his wife Olga, I owe my deepest thanks for their never-ending support and help in life.

I save my warmest and most loving thoughts for my dear children, Nea and Santeri. With the two of you, life is a beautiful experience. You have shown me what is most precious.

Jaana Koivunen

Turku, september 2011

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