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AMYLOID IMAGING IN ALZHEIMER'S DISEASE AND MILD COGNITIVE IMPAIRMENT BY POSITRON EMISSION TOMOGRAPHY

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Tillägnat min älskade familj
– utan kärlek dör människan och med den vetenskapen

ABSTRACT

Effectiveness of future treatment strategies in Alzheimer's disease (AD) will rely on early detection of disease and the possibility to clearly evaluate their effects. The findings presented in this thesis concerns both early *in vivo* detection of amyloid deposition in the brains of patients at risk of developing AD and the longitudinal changes of pathological and functional parameters in patients with AD.

A couple of years ago the first study with *in vivo* detection of amyloid using the radiotracer *N*-methyl [^{11}C] 2-(4′-methylaminophenyl)-6-hydroxy-benzothiazole also known as ^{11}C-Pittsburgh Compound B (^{11}C-PIB) with positron emission tomography (PET) was performed in collaboration between researchers in Pittsburgh, USA, Uppsala and Stockholm, Sweden. This first study showed a significant difference between AD patients and healthy controls in regards to their ^{11}C-PIB retention. The research presented in this thesis is both continuations and new investigations based on this initial research.

Results obtained in the studies presented in this thesis showed that the amyloid deposition is an early event in the development of AD present already in patients with mild cognitive impairment (MCI) that later develop AD. We could also show that the amyloid deposition in brain was closely correlated to concentrations of pathological biomarkers in the cerebrospinal fluid (CSF) at an early stage. While functional decline with decreased cerebral metabolic rate of glucose (CMRglc) measured with ^{18}F-FDG PET and episodic memory tests did not show a relationship in MCI patients but did at the clinical stage of AD. Our results also suggests that the early deposition of amyloid increase to a certain level and then reaches a plateau as shown by a quite stable ^{11}C-PIB retention in AD patients followed for a mean period of 2.5 years. We could also show that the dynamic ^{11}C-PIB PET scan do not only contain information on amyloid load but also information on brain function as the early frames contain a blood flow component that is related to CMRglc.

The general conclusions to be drawn from these studies are that ^{11}C-PIB PET shows promising results of both early detection of disease and the possibility to use it for evaluation of current and future anti-amyloid therapies. The possibility to extract functional information will further increase the usefulness of ^{11}C-PIB PET in AD research and clinical assessment of dementia.

LIST OF PUBLICATIONS

I. Henry Engler, **Anton Forsberg**, Ove Almkvist, Gunnar Blomquist, Emma Larsson, Irina Savitcheva, Anders Wall, Anna Ringheim, Bengt Långström, Agneta Nordberg. Two-year follow-up of amyloid deposition in patients with Alzheimer's disease. Brain. 2006;129:2856-2866

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IV. **Anton Forsberg**, Ove Almkvist, Henry Engler, Anders Wall, Bengt Långström, Agneta Nordberg. Amyloid deposition is an early event in AD showing complex relationships with CSF biomarkers and functional parameters. Submitted manuscript

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SAMMANFATTNING PÅ SVENSKA

Alzheimers sjukdom (AD) är den vanligaste formen av demenssjukdomarna och drabbar ungefär 6 procent av befolkningen över 65 års ålder. Idag är diagnostik av AD grundat på klinisk utvärdering främst genom kognitiva och neuropsykologiska test. De patologiska förändringar som karakteriserar AD är ansamlingar av intracellulärt hyperfosforylerat tau och extracellulära amyloida plack samt nedbrytning av hjärnans nervceller. Dessa förändringar i hjärnan kan idag studeras i levande patienter genom olika avbildningstekniker som magnetröntgen och positronemissionskamera (PET). PET kan användas kliniskt för att studera funktionella förändringar i hjärnan som försämringar av glukosmetabolism och blodflöde i hjässloben (parietallob) och tinningsloben (temporallob). Ganska nyligen utvecklades en ny molekyl för att mäta de amyloida placken i hjärnan kallad ^{11}C-PIB. Genom ^{11}C-PIB-PET har det visats att det går att separera AD-patienter från friska kontroller (FK) baserat på ^{11}C-PIB-bindningen i hjärnan. Hjärnans funktion kan studeras med PET genom att mäta glukosmetabolismen (CMRglc) med molekylen ^{18}F-FDG.

Målet med denna avhandling var att med hjälp av användning av PET utveckla och förbättra tidig diagnostik för Alzheimers sjukdom genom att undersöka tidiga neurofysiologiska och patologiska förändringar i hjärnan hos patienter med mild kognitiv svikt (MCI) och longitudinellt studera AD-patienter.

I min första studie genomförde vi en uppföljning av AD-patienter över ca två år med ^{11}C-PIB-PET och kunde då visa att ^{11}C-PIB-bindningen var tämligen stabil medan CMRglc i hjärnan och kognitionen fortsatte att försämras hos patienterna. Detta kan vara ett tecken på att amyloid-inlagringen når en platå medan hjärnans funktion fortsätter att progressivt försämras.

I den andra studien genomförde vi PET-undersökningar av amyloid och CMRglc i MCI patienter. MCI ses som ett förstadium till AD där ca 10-15 procent konverterar till AD under en ettårsperiod. Vi visade att 11 av 21 undersökta MCI-patienter hade förhöjda värden av amyloid i hjärnan och bland dessa konverterade 7 till AD. Detta pekar på att ^{11}C-PIB-PET kan vara en bra teknik för att på ett tidigt stadium urskilja de personer som med stor sannolikhet kommer att utveckla AD.

Den tredje studien undersökte möjligheten att använda tidiga delen av den dynamiska ^{11}C-PIB-PET-undersökningen som ett mått på blodflödet i hjärnan. Vi fann att detta mått var väl korrelerat till CMRglc och att dessa två parametrar visade samma resultat i gruppjämförelser (AD, MCI, FK). Detta tyder på att ^{11}C-PIB-PET skulle kunna användas för att få fram både patologisk och fysiologisk information.

I den fjärde studien undersökte vi relationerna mellan amyloid och glukos-metabolism i hjärnan, samt patologiska förändringar i cerebrospinalvätskan (CSF), och episodiskt minne. Vi kunde då visa att det fanns klara skillnader i dessa relationer i olika stadier av sjukdomen där amyloid i hjärnan var korrelerat till amyloid i CSF tidigt i sjukdomsprogressen medan funktionella mått som CMRglc i hjärnan och episodiskt minne var relaterade hos kliniskt diagnostiserade AD-patienter.

Slutsatsen i denna avhandling är att avbildningstekniken PET är en metod som visar stor potential att, med radioliganden ^{11}C-PIB, kunna användas för tidig diagnostik av patienter med stor risk att utveckla AD, studera sjukdomsutveckling och utvärdera nuvarande och kommande behandlingsstrategier.

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LIST OF ABBREVIATIONS

^{11}C-PIB	*N*-methyl [^{11}C] 2-(4´-methylaminophenyl)-6-hydroxy-benzothiazole
^{18}F-FDG	[^{18}F]-2-deoxy-d-glucose
Aβ	β-Amyloid
ACh	Acetylcholine
AChE	Acetylcholinesterase
AChEI	Acetylcholinesterase inhibitor
AD	Alzheimer's disease
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
CBF	Cerebral blood flow
CMRglc	Cerebral metabolic rate of glucose
CSF	Cerebrospinal fluid
MCI	Mild cognitive impairment
MMSE	Mini mental state examination
MRI	Magnetic resonance imaging
nAChR	Nicotinic ACh receptor
NFT	Neurofibrillary tangle
NP	Neuritic plaques
PET	Positron emission tomography
SPECT	Single photon emission computed tomography
SPM	Statistical parametric mapping
SUV	Standard uptake value

1 INTRODUCTION

1.1 DEMENTIA

Dementia is the third most common cause of death after cancer and cardiac vascular disorders, and Alzheimer's disease (AD) is the most common form of dementia. Other dementias include vascular and frontotemporal lobe dementia, dementia with Lewy bodies, and secondary dementias such as in Parkinson's disease. The estimated cost of dementia worldwide was in a recent study calculated to 315.4 billion USD with a prevalence of 29.3 million demented patients [1]. It has further been estimated that the number of AD patients will have quadrupled by 2050 [2]. With such a devastating disease for the suffering patient, family/friends, and society, there is a great need for development of diagnostic tools for earlier detection and differential diagnosis of dementia, as well as better treatment options than currently available.

1.2 ALZHEIMER'S DISEASE

AD is the most common form of dementia characterized by symptoms such as progressive loss of memory and other cognitive functions. The pathological hallmarks of AD are degeneration of cholinergic neurons, extracellular deposits of neuritic plaques (NPs), constituted mostly of β-amyloid (Aβ) [3, 4], and intracellular neurofibrillary tangles (NFTs) formed by hyperphosphorylated tau (Figure 1) [5]. Other pathological changes include loss of neurons, synapses and dendritic dearborization.

AD can be divided into two categories, sporadic/late onset AD and familial/early onset AD. In familial AD a number of different mutations in genes, involved in production of Aβ, have been found to be directly responsible for the etiology of disease. These findings have led to the amyloid cascade hypotheses that is thought to be involved also in sporadic AD [6]. The genes involved in amyloid production and the implications in AD will be discussed below.

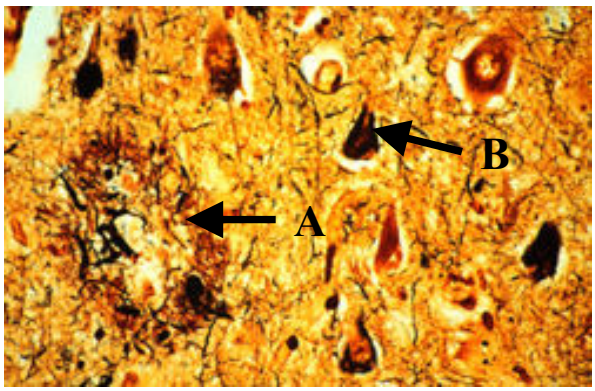

Figure 1. The pathological hallmarks of AD including the amyloid plaques (A) and the neurofibrillary tangles (B).

1.2.1 Genetics

The discovery of mutations in specific genes shown to be the direct cause of disease in the familial form of AD has shed a lot of light on the probable etiology of also the sporadic form of AD [6]. The genes in question are amyloid precursor protein (APP, chromosome 21) [7-10], Presenilin 1 (chromosome 14) [11], and Presenilin 2 (chromosome 1) [12] (Figure 2). The phenotypes coupled to these genotypes are, among others, increased production and/or deposition of Aβ-peptides, and augment in the more aggregating form $A\beta_{1\text{-}42}$ [13]. The Aβ production is seen in the APP processing cascade in Figure 2.

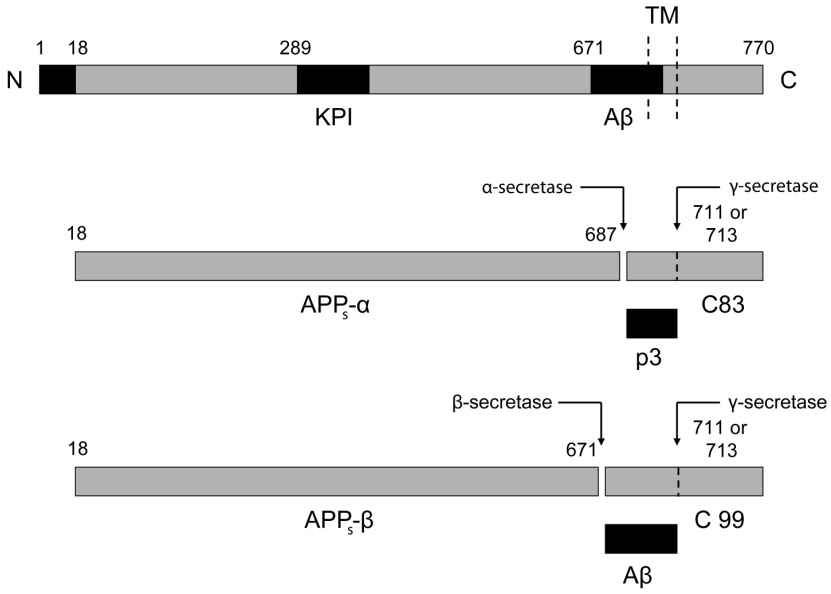

Figure 2. Aβ is produced from proteolytic cleavage of APP by β- and γ-secretases, called the amyloidogenic pathway.

Also in sporadic AD genetic components is thought to have a great influence on the risk of developing disease with one recent study suggesting that development of AD might be up to 48 percent reliant on genetic factors [14]. One genetic risk factor is the ε4 allele of Apolipoprotein E (ApoE-ε4, chromosome 19) [15]. APP transgenic mice deficient of ApoE show reduced Aβ deposition suggesting an involvement of ApoE in the pathologic processes of AD [16]. The association between ApoE-ε4 and AD is thought to be through ApoE's involvement in lipid metabolism [17]. ApoE is a regulator of lipid metabolism such as cholesterol, which has been associated with AD in both epidemiological and biochemical studies [18]. The relationship between cholesterol and AD is dualistic in nature. Cellular cholesterol level regulates synthesis of Aβ, and Aβ can in turn regulate the cholesterol level, which is involved in several AD related processes such as modulation of tau phosphorylation, synapse formation and function, and neurodegenerative processes [18]. The hypothesized involvement of cholesterol in AD pathology has resulted in studies of the possibility to prevent and/or treat AD with cholesterol lowering drugs, but none have so far shown any conclusive beneficial results [18].

1.2.2 Neuropathology

The actual cause of AD is still a mystery, although a lot of its pathological and neuropsychological characteristics have been thoroughly studied. The morphological findings and genetic linkage have given rise to the theories of possible pathological pathways including the amyloid cascade theory [6]. This theory postulates that an imbalance between the removal and production of Aβ leads to its progressive accumulation, which starts a series of processes leading to synaptic dysfunction, microgliosis and neuronal loss, clinically manifested by memory loss and impaired cognitive function. The pathological changes including deposition of amyloid aggregating into NPs and formation of NFTs probably begin many years before cognitive symptoms arise [19, 20]. The progress of the disease has been divided into 6 stages (I-VI) based on the temporal distribution of NFTs [21]. The pathological changes begin in the medial temporal lobe including entorhinal cortex and hippocampus and progress from there to paralimbic, basal temporal cortex and then to other neocortical association areas [22]. Based on the distribution of amyloid plaques three stages of temporal and regional distribution have been proposed originating in the orbitofrontal and temporal cortices, distribution becomes denser, spreads to parietal cortex and finally throughout the neocortex. Post mortem studies have found that density of NFTs in the medial temporal cortex is related to memory performance [23, 24] while the density of amyloid plaques is not [24]. Studies *in vivo* are needed to elucidate the true relationship between amyloid and tau pathology and memory performance.

The Aβ peptide that constitutes the amyloid plaques is a self-aggregating, 39 to 43 amino acid metalloprotein product derived from the proteolytic cleavage of APP by β- and γ-secretases (Figure 2) [13, 25]. The Aβ aggregates from soluble form to insoluble fibrils wrapped around each other, forming β-pleated sheets [26]. The plaques are closely surrounded by dystrophic axons and dendrites, reactive astrocytes and activated microglia [27]. Several *in vitro* and *in vivo* studies have shown Aβ to be directly toxic to neurons, leading to the aggregation of amyloid plaques and secondary hyperphosphorylation of the tau protein [26]. The diffuse Aβ deposits are the earliest forms of changes found in AD, but these can also be seen in normal aging individuals suggesting that these forms might be nontoxic depositions [28]. It is not known exactly which forms of Aβ execute the neuronal toxicity and which forms that should be the aim for therapeutic intervention [29]. Synaptic dysfunction might be caused by subtle changes in soluble oligomers of Aβ [30], which also seems to correlate better with cognitive function than amyloid plaques [31]. As amyloid depositions are present in brain regions involved in memory and cognition preceding other disease related symptoms suggests that these processes occur in presymptomatic stages of the disease. Methods able to show these changes might therefore serve as good diagnostic tools [26]. NFTs are intracellular bundles of paired helical filaments of hyperphosphyralated tau proteins [26]. The hyperphosphorylation of tau reduces its ability to bind microtubules leading to cytoskeletal degeneration and cellular death [32]. Most of the neurotransmitter systems in the brains are affected in AD, but the cholinergic system is most affected with reductions of cholinergic neurons especially in the hippocampus and nucleus basalis [33].

The course of the disease can today be monitored *in vivo* by functional changes in brain, such as glucose metabolism or blood flow. This is based on the relationship

between brain activity and synaptic transmission that is highly energy consuming. The loss of, and/or reduced activity of synapses and neurons is reflected by decreased glucose metabolism and blood flow assessable by neuroimaging techniques which will be discussed below.

1.2.3 Cognitive and neuropsychological changes

The disease course in AD seems to follow a certain sequence of cognitive decline with the initial deterioration thought to be affecting episodic memory [34, 35]. This preclinical phase is now commonly referred to as mild cognitive impairment (MCI) and is discussed in detail below [36]. At the diagnostic and clinical stage of AD other cognitive functions deteriorate including verbal and spatial cognition, executive function, and complex attention [34, 35]. Reaching the fully developed stage of AD also short-term memory, perception and motor function become impaired [34, 37, 38]. In the last stages of the disease progression psychological changes can also be present including mood changes, depression and hallucinations.

1.2.4 Clinical diagnosis

The clinical criteria for Alzheimer's disease are formulated in the guidelines of the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS–ADRDA) [39]. The definite diagnosis of AD is only possible postmortem, when the classical morphology can be assessed. The diagnosis of probable dementia, including AD, is done through several assessments and by excluding other possible causes of memory decline. The tests that are available in an academic hospital setting are as follows: neuropsychological investigation, functional assessment and structural imaging including magnetic resonance imaging (MRI) and/or computerized tomography (CT). At specialized research oriented hospitals additional methods for dementia diagnosis might be available including functional imaging including positron emission tomography (PET) and/or single photon emission computed tomography (SPECT), blood chemistry and analysis of pathological biomarkers in cerebrospinal fluid (CSF). Using these techniques research has lead to a better diagnosis of AD than was possible for a couple of years ago and it was recently proposed how the NINCDS-ADRDA criteria could be modified [40].

Dementia is characterized by loss of memory and or more cognitive abilities, namely: aphasia (language, spoken and written), apraxia (voluntary movements, i.e. dressing), agnosia, (sensory input, i.e. identifying objects by sight) or disturbance in executive functions. It should also be a substantial impairment in social and occupational functioning. Probable Alzheimer's disease as defined by the NINCDS-ADRDA criteria is diagnosed if there is an insidious onset of symptoms with cognitive loss documented by neuropsychological tests and a gradual progression. There should be no physical signs, neuroimaging or laboratory evidence for other diseases that could cause dementia (such as, stroke, Parkinson's disease, subdural hematomas, or tumors) [39].

1.2.5 Mild cognitive impairment

Mild cognitive impairment (MCI) is thought to be a transitional phase before progression to AD, and patients with MCI have an increased risk of developing AD [41]. About 10-15 percent of patients diagnosed as MCI convert to AD over a one year period compared to about 1-2 percent in the normal elderly population [41]. The accuracy of the diagnostic criteria used to determine MCI is today low to moderate [42]. The diagnosis is quite indeterminate but the common criteria used as defined by Petersen and collaborators are: memory complaint; objective memory impairment; preservation of general cognitive functioning; no or minimal impairment of daily life activities; and not fulfilling the criteria for dementia [43]. MCI is often subclassified into amnestic MCI having memory deficits and nonamnestic MCI not having memory deficits but other cognitive problems. Both these classifications can be of either single domain type with just one cognitive deficit or multiple domain type involving several cognitive domains.

A great deal of research is performed to find biological measurements, possible to study *in vivo,* that could aid in the diagnosis of MCI patients with a high risk of developing AD. Some of the methods with this aim including neuroimaging and CSF biomarkers are discussed below.

1.3 CSF ANALYSIS IN AD

The use of biomarkers in CSF such as $A\beta_{1-42}$, total and hyperphosphorylated tau (tTau and pTau) have been shown to have prognostic value in predicting which MCI patients will develop AD [44-47]. This suggests that these measurements might be useful in earlier diagnosis of AD. It has also been shown that combining different methods such as MRI and CSF measurements give higher sensitivity and specificity in retrospective studies of conversion from MCI to AD [48]. Two recent longitudinal studies have shown both short and long term stability in intraindividual levels of CSF biomarkers [49, 50]. This stability was shown by highly significant correlations between the baseline and follow-up measurements for $A\beta_{1-42}$, tTau and pTau [49, 50]. It was also shown that the levels of CSF biomarkers in MCI patients that converted to AD had significantly higher $A\beta_{1-42}$ and lower pTau and tTau levels compared to stable MCI patients and controls [49].

1.4 NEUROIMAGING IN AD

In the last decade the use of neuroimaging has greatly increased as a tool for diagnosis, evaluation of treatment, and AD research. PET, MRI and SPECT are now greatly appreciated methods and results from neuroimaging studies have generated new insights about pathological and functional changes in the etiology and progression of AD.

1.4.1 Magnetic resonance imaging

As described before, structural MRI is today used in the diagnosis of AD to exclude other possible causes of dementia such as vascular lesions, tumors or hydrocephalus. Research studies in AD patients using structural MRI have shown significant atrophy due to neuronal deterioration in the medial temporal lobe, especially the hippocampus

and entorhinal cortex [51, 52]. The degree of cerebral atrophy in the hippocampus measured *in vivo* with MRI has been shown to correlate with the neurofibrillary pathology staging [53, 54]. These specific changes have been shown early in the course of the disease and it is thought that it might be possible to use MRI for discriminating MCI patients with high risk of developing AD [55, 56].

1.4.2 Positron emission tomography

By the development of the PET technique it is now possible to do *in vivo* measurements of cerebral glucose metabolism (CMRglc), cerebral blood flow (CBF), receptor density, enzymatic activity and inflammatory processes. PET utilizes positron emitting radionuclides with short half-lives, produced in a cyclotron, such as ^{11}C (~20 min), ^{13}N (~10 min), ^{15}O (~2 min) and ^{18}F (~110 min). The respective radionuclide is chemically attached to the specific tracer molecule producing a radiotracer. The radiotracer is injected into the subject and distributed by the blood to the tissue and the tracer's target. The radionuclide undergoes β-decay emitting a positron that is annihilated by an electron producing two 511 keV photons at almost 180 degrees to each other. These photons are detected by a scintillator detector ring and the location of the decay can be computed, thereby being the foundation of the possibility to quantify the amount of tracer in a given tissue. An overview of tracers used in AD research to study both functional changes and pathological hallmarks of the disease are listed in Table 1. Studies using some of these tracers will be described below and the findings in relation to AD will be discussed.

Table 1. Overview of PET tracers used in AD research

Glucose metabolism: ^{18}F-FDG
Cerebral blood flow: ^{15}O-H_2O
Cholinergic system
AChE: ^{11}C-PMP, ^{11}C-MP4A
Nicotinic receptors: ^{11}C-nicotine, ^{18}F-A85380
Muscarinergic receptors: ^{11}C-NMPB, ^{18}F-FP-TZTP
Dopamine system
Dopa decarboxylation and vesicular storage: ^{18}F-fluorodopa
Dopa transporter: ^{18}F-/^{11}C-FP-CIT
D_2/D_3 receptors: ^{11}C-raclopride, ^{18}F-fallypride
Serotonergic system
5HT transporter: ^{11}C-DASB
$5HT_{1A}$: ^{11}C-WAY-100635, ^{18}F-MPPF
$5HT_{2A}$: ^{18}F/^{11}C-Altanserin, ^{18}F-setoperone
Inflammation
MAO-B (astrocytes): ^{11}C-$_L$-Deprenyl
Peripheral benzodiazepine receptors (microglia): ^{11}C-PK-11195
Amyloid: ^{11}C-PIB, ^{11}C-SB-13, ^{11}C-BF-227, ^{18}F-BAY94-9172
Amyloid and tau: ^{11}C-FDDNP

AChE=acetylcholine esterase, GABA=γ-aminobutyric acid

1.4.2.1 Cerebral glucose metabolism

[^{18}F]-2-deoxy-d-glucose (^{18}F-FDG) PET is readily used to evaluate deterioration in the brain present in AD by analyzing the glucose utilization by neurons [57-59]. Specific changes in depravation of CMRglc have been shown in the parietal and temporal cortices as well as posterior cingulum of AD patients with sparing of the cerebellum, sensory motor cortex and visual cortex [60, 61]. These specific changes found in AD have made ^{18}F-FDG PET a valuable tool in differential diagnosis of dementia where other dementia disorders show other patterns of cerebral metabolic decrease such as frontal and anteriotemporal deficits in frontotemporal dementia [62], and focal subcortical and cortical hypometabolism in patients with vascular dementia [63]. Global as well as parietotemporal decrease of CMRglc has also been shown in asymptomatic subjects from families with AD suggesting that hypometabolism might precede cognitive decline [64]. The use of neuroimaging as a potential diagnostic tool for early detection of AD has shown promising results in studies of MCI patients. Several studies suggest that decline in CMRglc can be used as a predictive marker to find MCI patients that later will develop AD [65-67]. The results of hypometabolism in MCI patients are nevertheless inconclusive with some studies showing large heterogeneity in CMRglc changes in this group of patients [68]. This might be due to compensatory mechanisms in the brain trying to manage pathological processes, and individual variations in the possibility to cope with the disease might explain differences in pathological burden needed to create cognitive deficits. It might therefore be important to combine findings from ^{18}F-FDG PET with other diagnostic markers such as brain atrophy [55], cognitive measurements [65, 68], and/or amyloid imaging [69] to increase diagnostic accuracy.

1.4.2.2 Cerebral blood flow

Similar results of specific impairments in parietotemporal brain regions and posterior cingulum as seen with ^{18}F-FDG PET have also been found studying CBF [70-73]. Both CMRglc and CBF have been suggested to be able to predict conversion from MCI to AD, although ^{18}F-FDG PET seems to be somewhat superior [74, 75]. Studies have also been able to show a clear relationship between CMRglc and CBF [73].

A recent study of the early frames of the dynamic ^{11}C-PIB PET includes a blood flow component that might be a good estimate of CBF and could possibly be used to extract functional information useful in both diagnosis and evaluation of treatment [76, 77].

1.4.2.3 Neurotransmitter systems

Studies of the cholinergic system have shown decreased acetylcholinesterase (AChE) activity in cortical brain regions especially the temporal lobe using the PET-tracers ^{11}C-PMP, ^{11}C-MP4A [78-81]. Research of nicotinic receptors has suffered from methodological problems with high unspecific binding for ^{11}C-nicotine but studies have still revealed decreased cortical binding in AD patients [82] that is related to performance in tests of attention [83]. No significant decrease in dopamine receptors has been seen in dementia diseases. An age related decline of dopamine D_2 receptors has been witnessed using ^{11}C-raclopride [84]. The serotonergic neurotransmitter system

is foremost interesting in depression which is one clinical symptom in later phases of AD and a decrease in $5HT_{2A}$ binding potential has been shown in AD patients [85, 86].

1.4.2.4 Inflammation

Activated microglia can be detected by using the peripheral benzodiazepine receptor ligand ^{11}C-PK-11195 [87] and elevated binding has been shown in AD patients [88]. The use of ^{11}C-PK-11195 as a diagnostic marker is unclear since elevated binding is observed in frontotemporal lobe dementia [89] and Huntington's disease [90]. It is nevertheless very important to study the role of inflammation in the development of AD and to do this in multi-tracer studies with amyloid imaging. New approaches to model the signal from ^{11}C-PK-11195 binding is needed to increase the use of the tracer and recent research shows that inclusion of a vascular component amplifies the binding potential in AD patients [91].

1.4.2.5 Amyloid imaging

Research concerning measurement of amyloid *in vivo* has been ongoing for about ten years. Different methods have been tried including monoclonal antibodies [92, 93], contrast agents for MRI [94, 95] and radiotracers for PET [96, 97]. In later years the possibility to measure amyloid *in vivo* has emerged by development of specific tracers for PET that binds to amyloid plaques [96, 98, 99]. Five PET tracers [^{18}F] 1,1-dicyano-2-[6-(dimethylamino)-2-naphtalenyl] propene (^{18}F-FDDNP) [99], *N*-methyl [^{11}C] 2-(4′-methylaminophenyl)-6-hydroxy-benzothiazole (^{11}C-PIB) [100], 4-N-methylamino-40-hydroxystilbene (^{11}C-SB-13) [98], 2-(2-[2-dimethylaminothiazol-5-yl]ethenyl)-6-(2-[fluoro]ethoxy)-benzoxazole (^{11}C-BF-227) [101], and trans-4-(*N*-methylamino)-4′-(2-[2-(2-[11F]fluoro-ethoxy)- ethoxy]-ethoxy)-stilbene (^{18}F-BAY94-9172/11F-AV1/ZK) [102] have so far been tested patients (Figure 3).

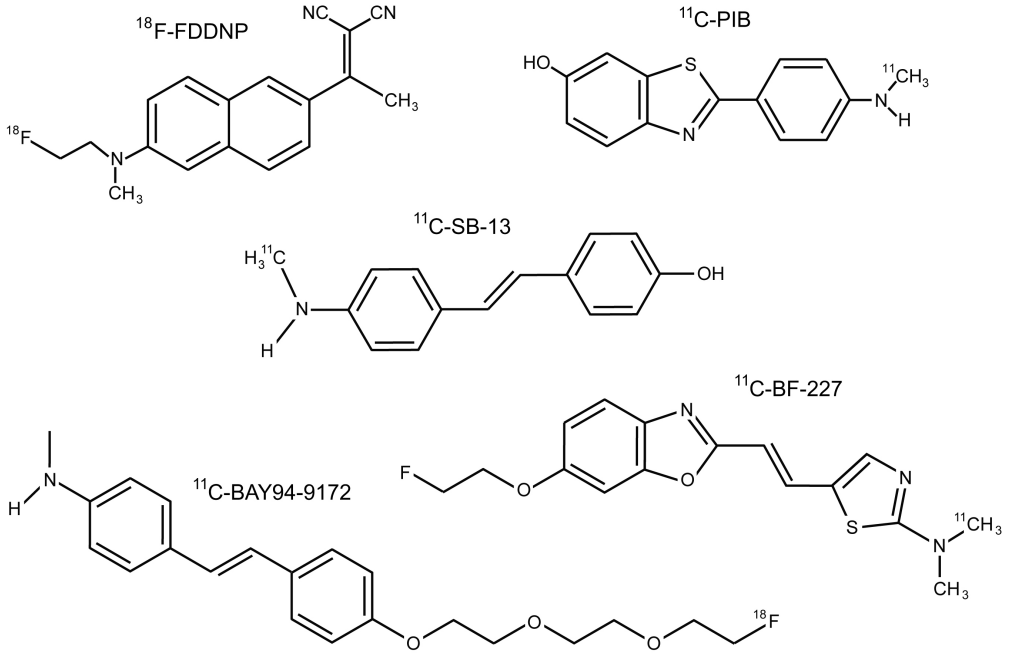

Figure 3. Amyloid imaging molecules that have been used *in vivo* with PET.

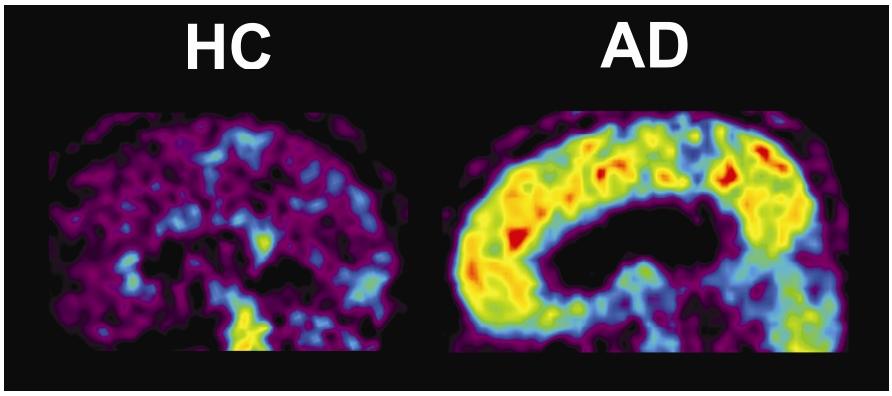

Figure 4. Example of ^{11}C-PIB retention in an old healthy control (HC) and a patient with Alzheimer's disease (AD),

^{11}C-PIB is a thioflavin-T derivate, and one of the most promising *in vivo* imaging techniques that has been developed so far. ^{11}C-PIB has been showed to bind amyloid with high affinity and cross the blood brain barrier to a sufficient degree [96, 103-105]. One high affinity binding site with a B_{max} of 1407 pmol/g with K_d of 2.5 and a low affinity binding site with a B_{max} of 13000 pmol/g and K_d of 250 nM have been found for ^{11}C-PIB [106].

Animal studies have shown that ^{11}C-PIB crosses the blood brain barrier and is cleared from normal brain tissue [107]. The results of binding studies in autopsy-derived human brain tissue suggested that ^{11}C-PIB is a relevant ligand for the measurement of amyloid deposition but not for the detection of neurofibrillary tangles, since ^{11}C-PIB mainly binds to aggregated fibrillar Aβ deposits [108]. It has been proposed that ^{11}C-PIB might preferably bind to the form of Aβ truncated at position 3 ($Aβ_{1-42}3$(pE)) [109]. $Aβ_{1-42}3$(pE) is an Aβ species that has been associated with accelerated formation of amyloid plaques [110, 111]. It has recently also been shown that ^{11}C-PIB binding *in vivo* correlates well with ^{11}C-PIB autoradiography to fibrillar forms of Aβ in post mortem brain tissue from the same patient [112]. In line with these findings the ^{11}C-PIB retention measured with PET correlates with the Aβ aggregates from frontal cortical brain biopsy specimen [113]. There have been some problems using ^{11}C-PIB in *in vivo* studies with transgenic mice models of AD [106, 114]. This issue was recently solved by using a high-specific radioactivity of ^{11}C-PIB showing increased activity with age [109]. It was also evident that it might be possible to evaluate new anti-amyloid treatment strategies in mice using ^{11}C-PIB and micro PET as a decrease in retention was found after administration of and anti-Aβ antibody for passive immunization [109].

In the first *in vivo* study in humans in Uppsala, Sweden it was found that ^{11}C-PIB showed high retention in the frontal and temporal-parietal cortices and striatum in AD patients compared to healthy controls [100]. The retention of ^{11}C-PIB was similar in AD patients and healthy controls in brain regions known to be relatively unaffected by amyloid deposition, such as the pons, cerebellum and subcortical white matter [100]. Examples of ^{11}C-PIB retention in an AD patient and a healthy control are seen in Figure 4. The significant differences between AD patients and healthy controls have been replicated using different means of data quantification as well with both regions of interest (ROI) based and voxel based evaluation methods [115-117]. ^{11}C-PIB binding

has been reported in a small number of healthy controls [100, 118, 119] and longitudinal studies are needed to find out if these patients are in a prodromal stage of AD. Nondemented older subjects with high ^{11}C-PIB retention have shown greater decline in CBF in certain brain areas compared to subjects with low ^{11}C-PIB retention suggesting a decrement in neuronal function [120]. The study also showed some areas with increased CBF suggesting compensatory mechanisms to cope with the pathological strain of amyloid deposition [120]. Some studies have reported a relationship between high ^{11}C-PIB retention and low CMRglc in AD patients [100, 121]. One study found a relationship between gray matter atrophy measured with MRI and the amyloid burden measured with ^{11}C-PIB PET [122], while another study failed to find any relationship between degree of atrophy and amyloid load measured post mortem [123]. The topographical distribution of amyloid depositions measured with ^{11}C-PIB PET has been related to the default network and it is hypothesized that there might be connections between neuronal activity and pathological changes in the development of AD [124]. Relationship between levels of ^{11}C-PIB binding and levels of pathological biomarkers in CSF has been found, with a somewhat stronger correlation between ^{11}C-PIB binding and $A\beta_{1-42}$ than with tTau or pTau [125, 126]. ^{11}C-PIB PET might be promising as a diagnostic marker as it is possible to find ^{11}C-PIB positive MCI patients [115, 127, 128]. These studies have found, in MCI patients as a group, intermediate levels of ^{11}C-PIB binding compared to healthy controls and AD patients.

Retention of ^{11}C-PIB seems to be quite AD-specific, with higher levels in AD compared to patients with frontotemporal lobe dementia [127, 129] and Parkinson's disease [127, 130]. Patients with Lewy body dementia have shown high but variable ^{11}C-PIB retention in the brain [127, 131] and this binding is thought to be caused by binding to amyloid and not to Lewy bodies [132]. Retention of ^{11}C-PIB has also been found in a small number of patients with late-life depression with co-occurring symptoms of MCI but not in depressed patients without any cognitive problems [133]. ^{11}C-PIB PET has been shown to be more accurate in diagnosis of AD compared to ^{18}F-FDG PET, with accuracies of 90 percent respectively 70 percent [134]. These studies suggests that ^{11}C-PIB PET might be a suitable method for early detection of AD pathology and differential diagnosis [135]. Nevertheless, prospective longitudinal studies are needed to elucidate the prognostic value of ^{11}C-PIB PET and its relationship to other pathological and functional parameters needs to be addressed to understand the temporal properties of AD related pathological changes.

^{18}F-FDDNP has been shown to bind Aβ in vitro at two binding sites, one with high affinity (0.12 nM) and one with lower affinity (1.9 nM) and binding studies in autopsy brain tissue have showed a B_{max} of 144 nM and a K_d of 0.75 nM [136, 137]. Binding to NFTs has also been shown for ^{18}F-FDDNP [138]. AD patients have significantly higher ^{18}F-FDDNP binding compared to healthy controls [99, 139], and MCI patients have intermediate levels of ^{18}F-FDDNP binding compared to AD patients and healthy controls [139]. ^{18}F-FDDNP binding correlates with CMRglc measured with ^{18}F-FDG PET and with cognitive measurements [139]. One recent study showed that ^{18}F-FDDNP retention showed somewhat different topographical distribution than ^{11}C-PIB including medial temporal brain regions while being less abundant in frontal cortex [140] consistent with previous findings of distribution patterns for amyloid plaques and NFTs [21]. Probes for selective imaging of tau pathology are also under development [141].

^{11}C-SB-13 has shown binding to post mortem AD brain tissue with a K_d of 2.4 nM and a B_{max} of 14-45 pmol/mg [142]. ^{11}C-SB-13 binding has been compared to ^{11}C-PIB and showed similar results regarding regional distribution, with increased retention in frontal and posterior temporal-inferior parietal association cortices in AD patients compared to healthy controls [98].

^{11}C-BF-227 has shown a K_i of 4.3 in binding studies with $A\beta_{1-42}$ fibrils [101]. ^{11}C-BF-227 has shown to have significantly higher cortical retention in AD patients compared to control subjects, especially in the basal portion of the frontal, temporal and parietal regions [101].

^{11}F-BAY94-9172 is the most recently developed PET tracer to be studied *in vivo* [102]. It has been shown to bind with high affinity to amyloid plaques with a K_i value of 6.7±0.3 to AD brain tissue [143]. The first study performed *in vivo* showed significant higher retention of ^{11}F-BAY94-9172 in cortical brain regions in AD patients compared to healthy controls and patients with frontotemporal lobe degeneration [102].

1.5 TREATMENT STRATEGIES

Treatment of mild AD today relies on symptomatic treatment of the cholinergic nervous system by AChE inhibitors (AChEI) such as donepezil, rivastigmine and galantamine [144]. By inhibiting the AChE the levels of acetylcholine (ACh) is elevated in the synaptic cleft which strengthens the neurotransmission resulting in positive effects on brain function. The three AChEI:s used today all have different pharmacological properties with diverse selectivity, inhibition and bioavailability [145]. Galantamine is thought to provide additional clinically beneficial effect by allosteric interaction with presynaptic and/or postsynaptic nicotinic ACh receptors (nAChR) [146].

Studies of treatment with AChEIs have shown both short- and long-term effects on cognitive measurements [147-155]. Beneficial effects on functional measurements of brain status, such as CMRglc and CBF, have been shown after treatment with donepezil [156] and rivastigmine [157-159].

Besides the AChEIs, the only treatment of AD approved is the noncompetitive NMDA-receptor antagonist memantine. Clinical studies in severe AD patients have shown beneficial effects on functions of daily living [153, 160] and that memantine can be delivered safely together with AChEIs [161].

There are several attempts on new treatment strategies of AD based on the amyloid hypotheses [6]. Research involves lowering production of Aβ by inhibiting γ- and β-secretase, preventing oligomerization and increasing clearance of Aβ [6]. Phenserine is a novel drug aiming to lower Aβ production and has recently reached human trials [162]. Beside its ChEI effect, phenserine treatment has shown reduction of Aβ levels by regulating the APP translation [162] in cell cultures and mice [163]. (+)-Phenserine reduces APP and increases neuronal differentiation of stem cells [164], as well as induce beneficial effects of cognitive measurements in rodents [165]. A recent study used ^{11}C-PIB PET and ^{18}F-FDG PET to evaluate long term treatment with phenserine in AD patients [166]. The AD patients showed positive effects on cognition after treatment and the changes in brain amyloid levels correlated with amyloid levels in CSF [166]. It was also found that CMRglc was increased and correlated with cognitive function and CSF levels of $A\beta_{1-40}$. Treatment with a γ-secretase inhibitor showed nonsignificant decrease in CSF levels of $A\beta_{1-40}$ that could be a sign of mild changes of

APP processing [167]. Intervening amyloid oligomerization by interaction with amyloid metabolites is thought to be the action of NC-531 currently in phase 3 studies [168]. One vaccination study in AD patients had to be discontinued because 17 out of 300 patients developed autoimmune encephalitis [169]. Novel approaches are being developed including passive immunization by injecting anti-amyloid antibodies.

2 AIMS WITH THE THESIS

With a devastating illness such as AD it is obvious that there is great need for tools for earlier diagnosis and monitoring of present and future treatment strategies. The aim of this thesis was to use ^{11}C-PIB PET to study amyloid deposition in the brains of AD and MCI patients and relate these findings to CSF biomarkers and functional changes of CMRglc measured with ^{18}F-FDG and cognition. Several more specific aims were introduced coupled to the four studies presented in this thesis.

- Explore the longitudinal changes in amyloid deposition measured with ^{11}C-PIB PET and relate these to functional changes in CMRglc measured with ^{18}F-FDG and cognitive performance.
- Investigate when amyloid deposition measured with ^{11}C-PIB PET begin in the development of AD by studying patients with mild cognitive impairment and the conversion to AD.
- Study the possibility to use the dynamic information of the ^{11}C-PIB PET scan to produce both functional estimates of blood flow from the early frames of the scan and pathological information of amyloid load by the late frames.
- Further explore the relationships between pathological changes in the brain by ^{11}C-PIB PET and CSF biomarkers, and functional changes in the brain by ^{18}F-FDG PET and neuropsychological tests, and how they differ in various brain regions and change during the progression from MCI to clinical AD.

3 SUBJECTS AND METHODS

3.1 GENERAL INFORMATION

All participants were recruited from the Department of Geriatric Medicine, Karolinska University Hospital Huddinge, Stockholm, Sweden, where they had undergone extensive assessment of memory problems. Dementia assessments included physical examination, evaluation of neurological and psychiatric status, cerebrospinal fluid tapping, blood analysis including ApoE genotyping, MRI and/or SPECT, neuropsychological testing and Mini-Mental-State Examination (MMSE). The AD patients were diagnosed as probable AD according to the NINCDS-ADRDA criteria [39]. Diagnosis of MCI was performed according to the modified Peterson criteria [41, 43]. CSF analysis were performed as described elsewhere [170].

3.2 NEUROPSYCHOLOGICAL ASSESSMENTS

The neuropsychological tests that are routinely used in the assessment of patients with memory problems at the Department of Geriatric Medicine and available for statistical analysis covered seven domains including: intelligence/global cognition; language; visuospatial ability; immediate memory; episodic memory; attention/cognitive speed and executive function [171]. Not all neuropsychological information was used in statistical analysis, the specific tests and the data used is described for each study separately.

3.3 POSITRON EMISSION TOMOGRAPHY

The tracers ^{18}F-FDG and ^{11}C-PIB were synthesized according to the standard good manufacturing process at Uppsala Imanet, and the method of synthesizing ^{11}C-PIB has been described in detail elsewhere [100, 104]. The PET scans were conducted using a whole body PET scanner, ECAT EXACT HR+ scanners (CTI PET-systems Inc.) with an axial field of 155 mm, providing 63 contiguous 2.46 mm slices with 5.6 mm transaxial and 5.4 mm axial resolution. The data was acquired in three-dimensional mode with a neuroinsert to reduce radiation originating outside the fields of view. The patients were scanned under resting conditions in a room with dimmed light and low ambient noise. The emission data was corrected for attenuation, random coincidences, dead time and scattered radiation. Image reconstruction was carried out by means of filtered back projection using a 6 mm Hanning filter. The ^{18}F-FDG PET scan had a duration time of 45 or 60 minutes divided into a number of frames ranging from 6 to 16. Parametric maps of CMRglc were created by the Patlak technique using the time course of ^{18}F-FDG in plasma as the input function [172, 173]. The ^{11}C-PIB PET scan had a duration time of 60 minutes with a number of frames of 15 or 24. Mean uptake values of the ROIs were obtained in the late time interval (40-60 min) and standard uptake values (SUV) were calculated by normalizing the data to patient weight and injected activity. The data were then normalized to the corresponding uptake in a reference region generating late scan ratio data (ROI/ref). The cerebellar cortex was chosen as reference region because of its previous reported lack of Congo red and thioflavin-S-positive plaques [174, 175].

3.3.1 Region of interest analysis

A standardized set of ROIs was used as described in detail by Engler et al, delineated using the Scanditronix program IDA [176]. All ROIs were paired for the right and left hemispheres, except for the pons. Cortical ROIs (1×3 cm) were defined in the frontal (three slices) and parietal (four slices) cortices. Cortical ROIs of the anterior part of the cingulum were defined four slices from the level of the thalamus and above, and the ROIs were linked, forming volumes of interest (VOIs). ROIs in the thalamus and cortical ROIs in the occipital and cerebellar cortices were defined at the level of highest uptake. At the level of the thalamus, ROIs surrounding the putamen were defined. Two circular ROIs (1.5 cm in diameter) were defined in the pons and then linked. A ROI in the white matter was defined at the level of the centrum semiovale. Regions in the sensory motor cortices and frontal association cortices were defined at a level 5 slices above the level of the thalamus. Cortical ROIs (0.7×3mm) were defined in the temporal cortex in two slices in each of the following regions: posterior/anterior lateral, inferior and medial (uncus). The ROIs in the consecutive slices were then linked to give a VOI for each area of the temporal cortex. A ROI including the whole brain was defined at the level of the thalamus. Two ROIs defining the posterior cingulum were drawn in two slices below the level of the thalamus and were then linked to create a VOI.

3.3.2 Statistical Parametric Mapping

Statistical Parametric Mapping 2 (SPM2) (The Wellcome Department of Behavioural Neurology, London, UK) and MATLAB 6.5 (The Mathworks) was used to perform voxel based analyses of PET data. PET images were first normalized into a standard stereotactic space [177, 178]. The ^{18}F-FDG images could be directly normalized using the PET template within SPM2. Images from the early frames of the PET scan were used to normalize the images of late scan ratio of ^{11}C-PIB retention. The normalization parameters were calculated using the early frame images and then applied to the late scan ratio images [179]. The images were then smoothed with an isotropic Gaussian filter (12-mm full width at half-maximum), and individual global counts were normalized by proportional scaling to the global mean of all voxels.

Analyses of differences in respective PET modality between groups were assessed by means of the model: “single subject, condition and covariates”, dividing the data into respective groups. Analysis of covariance between the PET measurements, concentrations of CSF biomarkers and episodic memory tests was performed by entering a regressor of respective covariate. Voxels were considered significant at a threshold of $p<0.001$, uncorrected. Clusters were considered significant at a threshold of $p<0.05$, corrected for multiple comparisons.

3.4 STUDY SPECIFIC INFORMATION

3.4.1 Paper 1

16 patients mild AD patients, 11 males and 5 females, with a mean age of 66.4±10.2 (range: 51-81) and a mean MMSE score of 24.3±3.7 previously recruited to the baseline study, had undergone ^{11}C-PIB and ^{18}F-FDG, at Uppsala PET-center. The results from the baseline study were published by Klunk, Engler et al [100]. All 16 patients and one healthy control were followed up after time period of 2.0±0.5 years (1.5 to 2.5 years) with ^{11}C-PIB and ^{18}F-FDG and neuropsychological investigations. The mean MMSE score for the 16 AD patients at follow-up were 22.7±6.1. Data from 6 healthy, aged-matched controls, studied at baseline, were utilized for comparisons. One of the healthy controls with high ^{11}C-PIB retention at baseline was reinvestigated.

Four additional AD patients (3 females and 1 male; 58–79 years of age; MMSE 9–28) were recruited to study the test/retest variability of ^{11}C-PIB PET. Three of the AD patients underwent two ^{11}C-PIB scans within 12 hours and the fourth patient after a 20-day interval. The test/retest variability was for each patient by means of the formula: Percent difference = ((R-T)/(R+T)) × 200; (T = test, R = retest). The mean absolute percentage difference was calculated for all four patients, yielding an interval of expected variance in analysis of PIB retention.

3.4.2 Paper 2

Twenty one MCI patients, 8 males and 13 females with a mean age of 63.3±7.8 year and a mean MMSE score of 28.2±1.4 were recruited and underwent ^{11}C-PIB and ^{18}F-FDG PET examinations at Uppsala PET-center. Neuropsychological investigations were done close in time to the respective PET scan. Neuropsychological data of episodic memory (Rey auditory verbal learning (RAVL) total learning, RAVL retention and Rey Osterrieth retention) was used for statistical analysis of group comparisons and correlations. Mean Z-scores for the episodic memory data were generated by relating the data to a reference material at Karolinska University Hospital Huddinge, Sweden [180].

During the study clinical follow-up was performed of MCI patients experiencing worsened cognitive status. 7 of the MCI patients were at follow-up considered to have converted to AD after a mean period 8±6 months after their respective PET scan. These patients were considered as converting MCI patients (MCI-c) and the remaining 14 were considered as nonconverting MCI patients (MCI-nc) in statistical analysis. Data from 27 AD patients, 13 males and 14 females with a mean age of 66.2±9.2 years and 6 healthy controls, 3 males and 3 females with a mean age of 67.3±8.8 years, were utilized as comparison material.

3.4.3 Paper 3

Thirty-seven AD patients with a mean age of 67.5±9.2 and a mean MMSE score of 23.7±4.0; and 21 MCI patients with a mean age of 63.3±7.8 and a mean MMSE score of 28.2±1.4 had previously undergone ^{11}C-PIB and ^{18}F-FDG PET scans.

Quantitative data of ^{11}C-PIB retention and CMRglc were generated as described above. Semi quantitative data of the early frames of the ^{11}C-PIB PET scan (ePIB) was

generated by summation of the frames from the first 5-6 minutes. SUV was calculated and the data was normalized to the reference value from the late scan SUV image.

3.4.4 Paper 4

^{11}C-PIB and ^{18}F-FDG PET data from 37 AD patients with a mean age of 67.5±9.2 and a mean MMSE score of 23.7±4.0; and 21 MCI patients with a mean age of 63.3±7.8 and a mean MMSE score of 28.2±1.4 was utilized for statistical analysis.

CSF data of $A\beta_{1\text{-}42}$, tTau and pTau, as well as neuropsychological data of episodic memory including RAVL total learning (RAVLtot) and RAVL retention (RAVLret) was correlated to the PET data to investigate relationships between the parameters in the total material of AD plus MCI and in AD patients and MCI subjects separately.

4 RESULTS AND DISCUSSION

This thesis is based on four papers investigating early and longitudinal pathological and functional changes in the course of AD by PET. The deposition of amyloid plaques in brain and the changes in CMRglc were investigated with ^{11}C-PIB- and ^{18}F-FDG PET respectively. In the first paper we could show that amyloid deposition was quite stable in patients while deteriorating shoed as decline in CMRglc and cognitive status. In the second paper we found that amyloid deposition is an early event, evident already in patients with just mild cognitive problems. It was also showed that all patients that converted to AD had high levels of amyloid already as MCI patients. The third paper of the thesis demonstrated that the early frames of the dynamic ^{11}C-PIB PET scan might be possible to use as a measurement of functional changes important for diagnostic purposes or evaluating treatment strategies. The fourth study illustrated that the pathological changes seen in brain with ^{11}C-PIB PET and in CSF biomarkers are closely related and are early markers of disease. The functional changes in rCMRglc and episodic memory performance were also closely related but seemed to decline after the pathological changes were evident.

4.1 PAPER 1 - IMAGING THE TIME COURSE OF AMYLOID DEPOSITION IN PATIENTS WITH ALZHEIMER'S DISEASE

Previous studies have shown that it is possible to image amyloid depositions in brain of AD patients by ^{11}C-PIB PET [100]. Post mortem studies have also shown that the pathological changes in amyloid load are not strongly related to cognitive status, while tau pathology shows stronger correlation to disease stage [22]. With this study we wanted to investigate the longitudinal changes in amyloid deposition measured with ^{11}C-PIB PET and relate that to functional changes in CMRglc measured with ^{18}F-FDG PET and neuropsychological investigations.

In a longitudinal study it is important to know what amount of change can be considered as significant and what variability the research method has. To investigate the variability in the ^{11}C-PIB PET method a test-retest study was performed. The results showed that the variability was about 4-7 percent in cortical brain areas.

We were able to reinvestigate all 16 AD patients and one healthy control with high ^{11}C-PIB retention at baseline. At a group level there were no changes in ^{11}C-PIB retention over the follow-up period in any cortical brain areas known to show high amyloid load ($p>0.05$) (Figure 5). Only the occipital cortex showed a small increase ($p<0.05$). On the contrast the glucose metabolism showed a significant decrease in all cortical brain regions ($p<0.01$) (Figure 5). When the patients were divided in slow progressing (AD-S) and fast progressing (AD-P) AD patients it was evident that the AD-P group had significantly higher ^{11}C-PIB retention than the AD-S group in posterior cingulum ($p<0.05$). Decrease in CMRglc was evident in both groups ($p<0.05$).

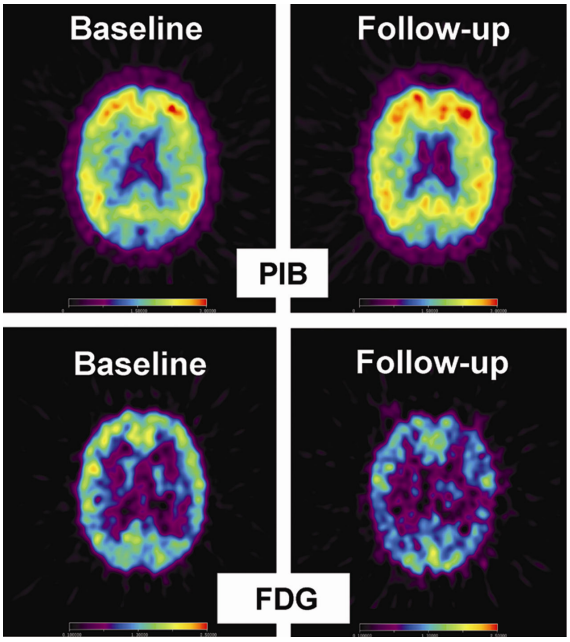

Figure 5. Representative images of ^{11}C-PIB retention and CMRglc in an AD patient showing quite stable ^{11}C-PIB retention and deterioration in CMRglc.

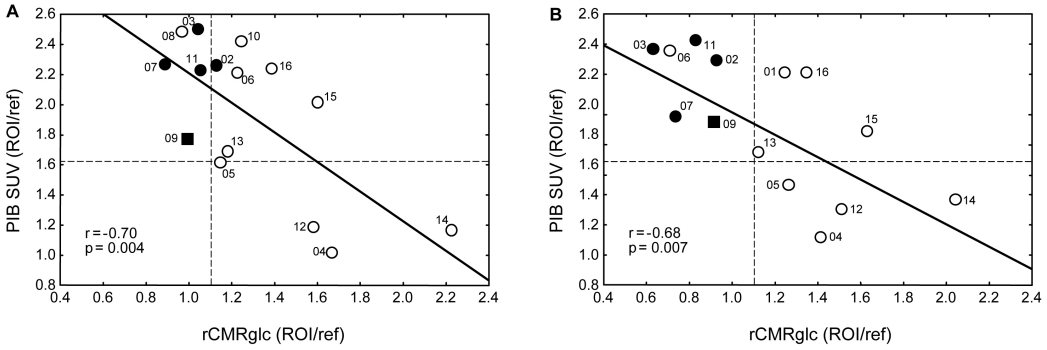

Figure 6. Correlation at baseline (A) and follow-up (B) between ^{11}C-PIB retention (ROI/ref) and rCMRglc (ROI/ref) in parietal cortex.

The results from analyses of covariance revealed a significant correlation between ^{11}C-PIB retention and relative CMRglc (rCMRglc) in the parietal cortex both at baseline (r=-0.70; p=0.002) and follow-up (r=-0.68; p=0.007) (Figure 6). ^{11}C-PIB retention correlated negatively with episodic memory and rCMRglc correlated positively with episodic memory in several cortical brain regions including the frontal, parietal cortices and posterior cingulum (p<0.05).

Our results showed that the amyloid load, measured with ^{11}C-PIB retention, were high in AD patients and seemed to be quite stable over time while the disease continued to progress with decrease in CMRglc and cognitive status. This stability in amyloid load has also been shown in studies of AD post mortem brain tissue [181] and transgenic mouse models of AD [182], as well as in CSF biomarkers [50]. There might also be dynamic changes in the formation and maturation of amyloid plaques that

influence the binding properties of ^{11}C-PIB. Two binding sites on amyloid-β fibrils have been found for ^{11}C-PIB [106]. The binding property of ^{11}C-PIB in vivo is hard to determine and it can not be excluded that structural changes during the evolution of the NPs could influence the binding of ^{11}C-PIB. It is also important to consider possible influence of AChEI therapy on amyloid deposition. All patients were on some AChEI drug treatment during the follow-up period and although no clear evidence of influence of these drugs on amyloid load has been found some studies suggest that it might be interactions that have an effect on the underlying pathological mechanisms of AD [183-185].

Our findings also indicate that there is a relationship between ^{11}C-PIB retention and disease state although the correlation between rCMRglc and cognitive status is stronger. The fact that ^{11}C-PIB-retention seems to be quite stable during the course of the disease suggests that ^{11}C-PIB PET could be a good method to evaluate new treatment strategies directed to decrease production or increase clearance of amyloid. Studies in patients with MCI and nonsymptomatic patients with familial forms of AD are needed to elucidate when the increase of amyloid occur. It is important to investigate when the accumulation of amyloid starts and its relationship to other pathological processes and onset of symptoms in order to know when treatment is needed to begin in order to have potential effect.

4.2 PAPER 2 - AMYLOID DEPOSITIONS IN PATIENTS WITH MILD COGNITIVE IMPAIRMENT

^{11}C-PIB PET has been shown to be a promising marker for detection of amyloid depositions in AD [100] and discrimination from other dementia related diseases such as frontotemporal lobe dementia [127, 129] and Parkinson's disease [127, 130]. Three studies, two with ^{11}C-PIB PET and one with ^{18}F-FDDNP PET, had before the publication of this study reported amyloid depositions in patients with MCI [115, 128, 139]. No study had in a large group of MCI patients investigated the ^{11}C-PIB binding and related it to cerebral glucose metabolism measured with ^{18}F-FDG PET and conversion to AD.

All 21 MCI patients underwent ^{11}C-PIB and ^{18}F-FDG PET and eleven of the patients showed high ^{11}C-PIB retention in cortical brain areas. The total group of MCI patients had significantly lower ^{11}C-PIB retention in cortical brain areas compared to AD patients. Seven out of the 21 MCI patients converted to AD during the study and all showed high ^{11}C-PIB retention similar to AD patients, significantly higher compared to healthy controls (Figure 7). There were no significant differences in rCMRglc between the converting MCI patients (MCI-c) and healthy controls and the nonconverting MCI patients (MCI-nc) showed significantly higher rCMRglc compared to the AD patients (p<0.01).

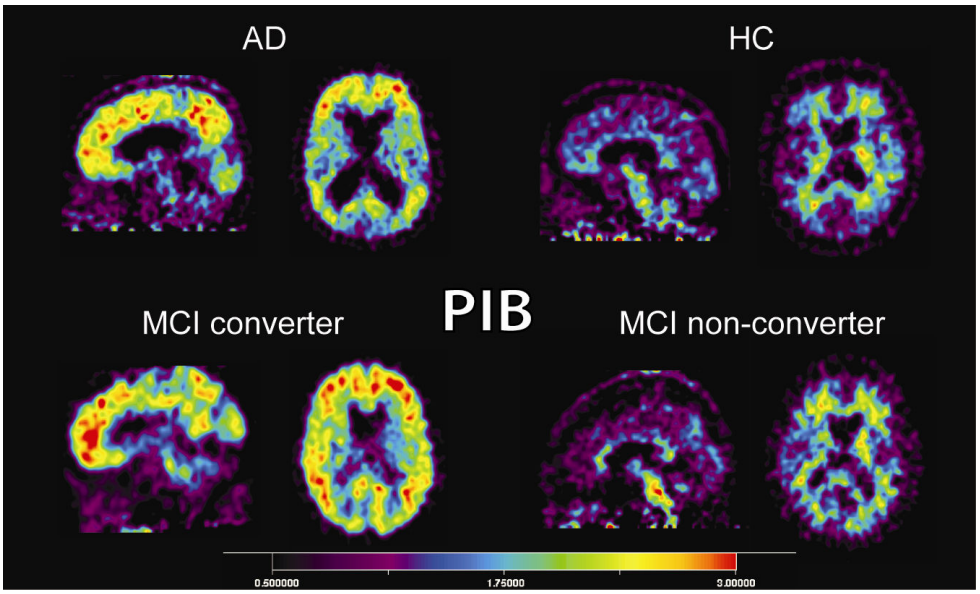

Figure 7. ^{11}C-PIB retention in one AD patient, one healthy control (HC), a MCI patient that later converted to AD, and one MCI that has not converted to AD.

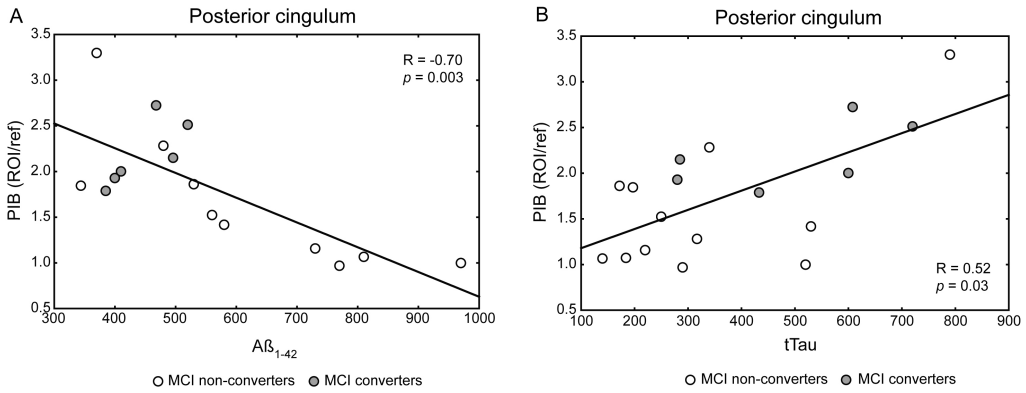

Figure 8. Correlation between ^{11}C-PIB retention vs $A\beta_{1\text{-}42}$ (A) and vs total tau (B) in MCI patients.

Significant correlations between ^{11}C-PIB retention and CSF measurements of $A\beta_{1\text{-}42}$ and tTau were found (Figure 8), while there were no significant correlations between ^{11}C-PIB retention and rCMRglc in any of the cortical brain regions. There was a weak correlation between ^{11}C-PIB retention, in posterior cingulum, frontal cortex and temporal cortex, versus episodic memory (p<0.05). No significant correlations were found between ^{11}C-PIB retention versus rCMRglc or rCMRglc versus episodic memory (p>0.05).

We aimed in this study to investigate ^{11}C-PIB retention in MCI patients to elucidate what percentage of such a heterogeneous group of patients would show ^{11}C-PIB retention and how that would compare to AD patients and healthy controls. We found that about half of the MCI patients were ^{11}C-PIB positive and about half ^{11}C-PIB negative. We also found that all MCI patients that later on converted to AD were ^{11}C-PIB positive and had levels similar to AD patients when only showing small cognitive deficits. It was also evident that the pathological changes of amyloid load measured in brain with ^{11}C-PIB PET was related to the biomarkers measured in CSF. This is in accord with findings by Fagan et al in a mixed subset of healthy controls and demented patients [125]. We did not find any clear decrease in rCMRglc in the MCI patients converting to AD as previously been described in other studies [66-68]. This discrepancy between our findings and other results might be due to the fact that our MCI patients are quite young with a more intact neuronal network and compensatory mechanisms trying to cope with disease causing processes [186]. One other possibility for quite normal levels of CMRglc in the cohort of MCI patients could be a different time course between the amyloid deposition and decline in CMRglc as suggested by our findings in the longitudinal study of AD patients [187].

Our results showed that ^{11}C-PIB PET of amyloid load in the brain might be promising as a diagnostic tool in determining MCI patients with an increased risk of developing AD.

4.3 PAPER 3 - BRAIN FUNCTION MEASURED BY ^{11}C-PIB PET IN AD AND MCI

The early frames of the dynamic ^{11}C-PIB PET include a blood flow component that might be usable to extract information about brain function [76, 77]. To be able to retrieve both functional and pathological information from a single dynamic PET scan would be valuable if ^{11}C-PIB PET will be established as a diagnostic tool and as a method to evaluate treatment strategies with reduced strain on the patient and costs involved.

Our results indicate that there are clear similarities between the decrease in rCMRglc and early frames of ^{11}C-PIB (ePIB) in AD compared to HC and MCI (Figure 9). The group differences in rCMRglc, measured by ^{18}F-FDG, were somewhat larger than with ePIB. Both parameters showed significantly lower values in AD patients compared to MCI patients in all cortical brain areas (p<0.05). Significantly lower rCMRglc in AD patients compared to healthy controls were found in frontal, parietal, occipital cortices and posterior cingulum (p<0.05). The posterior cingulum showed significantly lower value of ePIB in AD patients compared to healthy controls (p<0.05).

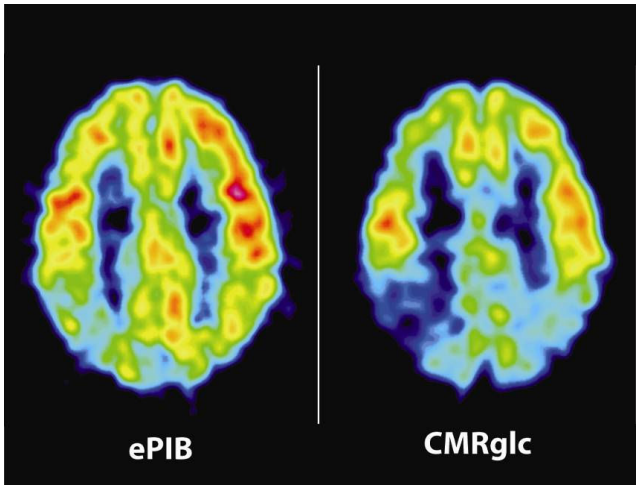

Figure 9. Example of ePIB and CMRglc in an AD patient showing clear deteroriation in parietal cortices.

Significant correlations in all areas analyzed were demonstrated between ePIB and rCMRglc suggesting that the early frames might be used as a surrogate to ^{18}F-FDG (p<0.05; Figure 10). No significant correlation between ePIB and ^{11}C-PIB retention was found in cortical brain regions (p>0.05). The only region that showed a significant correlation was the cerebral white matter (p<0.05). Significant correlations between rCMRglc and ^{11}C-PIB retention were found in the posterior cingulum, parietal and frontal cortices.

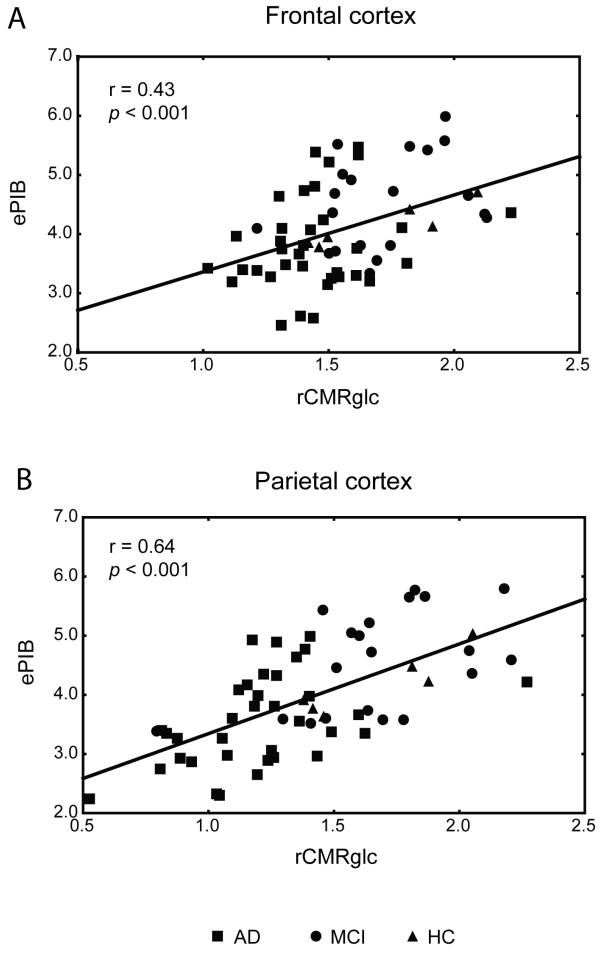

Figure 10. Correlation between ePIB and rCMRglc in AD, MCI patients and healthy controls (HC), showing the frontal (A) and parietal (B) cortices.

The results showed clear relationships between the cerebral glucose metabolism and ePIB as an estimate of CBF. The group comparisons also revealed similarities between the two functional measurements suggesting that the early frames of the dynamic ^{11}C-PIB PET scan might be used to give information on brain function and status. Studies using PET to determine CBF have shown significant decline in parietotemporal cortex and posterior cingulum [70]. These findings are in accord with the results in our study showing decreased ePIB in AD patients compared to healthy controls, reaching statistical significance in the posterior cingulum.

Combining neuroimaging techniques, including functional and structural imaging, with neuropsychological investigations and/or CSF biomarkers has proven valuable in order to increase diagnostic accuracy and will be necessary in future efforts to find people at risk of developing AD at an early stage [48, 55, 65, 68, 69]. Further studies are necessary to evaluate the diagnostic significance of CBF measured with the method presented in this paper as well addressing some methodological issues that needs further attention. The results are nevertheless promising in regards to the possibility to retrieve complementary information from a single dynamic ^{11}CPIB PET scan. Hopefully these new findings will prove useful in AD research, diagnostics and evaluation of new treatment strategies directed against amyloid.

4.4 PAPER 4 - AMYLOID DEPOSITION IS AN EARLY EVENT IN AD SHOWING COMPLEX RELATIONSHIPS WITH CSF BIOMARKERS AND FUNCTIONAL PARAMETERS

Earlier studies with ^{11}C-PIB PET have shown clear separation between AD patients and healthy controls [100] and high binding in converting MCI patients [188]. Some studies have shown relationships between ^{11}C-PIB binding versus cerebral glucose metabolism [100, 189], versus memory performance [189] and versus CSF biomarkers [125, 188]. No study has so far evaluated the relationships between these pathological and functional parameters in the same material comprised of patients from different stages of the disease process.

In this paper we wanted to further investigate the relationships between the pathological changes, measured in the brain with ^{11}C-PIB PET, and CSF biomarkers and functional changes in CMRglc measured by ^{18}F-FDG PET and neuropsychological tests of episodic memory. These relationships were assessed by looking at both temporal differences at different stages of the disease and regional differences between cortical and subcortical brain regions.

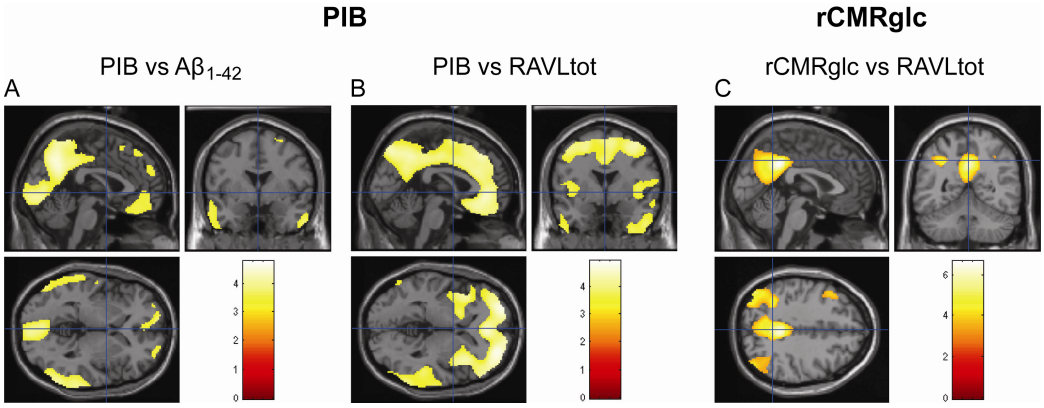

Figure 11. SPM analysis showing clusters with significant covariance between cerebral ^{11}C-PIB retention versus (A) levels of CSF $A\beta_{1-42}$ and versus (B) episodic memory scores measured by means of Rey Auditory Verbal Learning (RAVLtot) tests, as well as correlation between (C) rCMRglc and RAVLtot<0.001, uncorrected for multiple comparisons.

Retention of ^{11}C-PIB in AD brains was widespread (data not shown) and it correlated significantly with rCMRglc as well as with levels of CSF biomarkers, and episodic memory when the total material of AD and MCI patients was analyzed (Figure 11). The decrease in rCMRglc was more focally located to the parietal cortex and posterior cingulum compared with ^{11}C-PIB retention (data not shown), and the impairment of rCMRglc also showed a strong correlation with impairment in episodic memory in these brain areas (Figure 11). The ^{11}C-PIB retention and rCMRglc correlated significantly when data from the AD and MCI patients where analyzed together but this relationship was only found in the parietal cortex and posterior cingulum in AD patients alone and no significant correlation was found in MCI patients (Figure 12A). The ^{11}C-PIB retention correlated strongly to the CSF biomarkers when data from the

AD and MCI patients where analyzed together. The covariance between these measurements remained in the MCI patients but was lost in separate correlative analysis in the AD patients alone (Figure 12B). A significant relationship between ^{11}C-PIB retention and episodic memory was found in most brain regions in the whole material but only in posterior cingulum, left frontal cortex for AD patients alone and posterior cingulum [170], left frontal and temporal cortices (Figure 12C). Strong correlations between rCMRglc and episodic memory were found in all brain regions analyzed for AD plus MCI and AD patients alone but for MCI patients alone (Figure 12D).

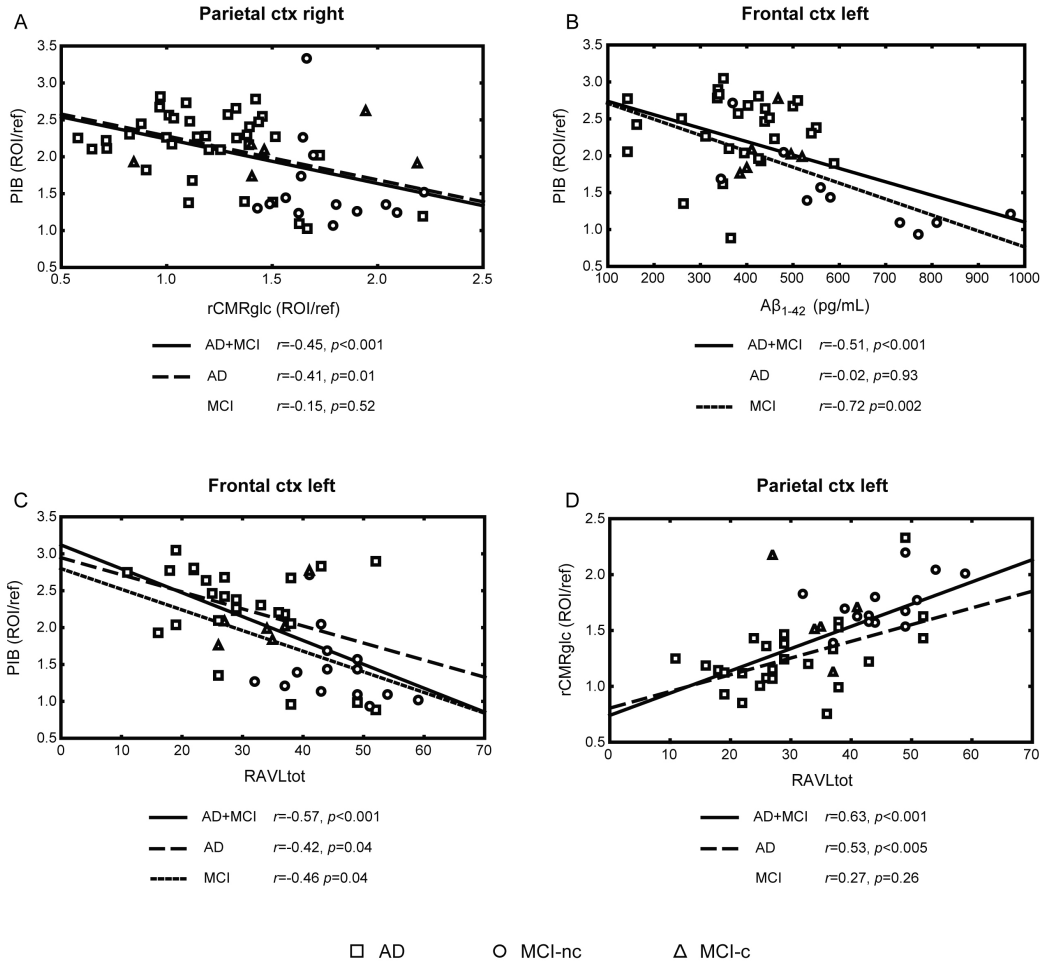

Figure 12. Correlations between ^{11}C-PIB retention versus rCMRglc (A), Aβ1-42 in CSF (B), versus RAVLtot score (C) and rCMRglc versus RAVLtot score (D).

Amyloid deposition measured with ^{11}C-PIB PET seems to be quite widely distributed in cortical brain regions which have been shown also in other PET studies using both ROI and voxel based methods [100, 117, 124]. This should be compared to the decreases in rCMRglc we found in the AD patients which were more focal in nature and involved areas known to be the most affected in AD, such as the parietotemporal cortex and posterior cingulum [60, 124, 190]. The topographical distribution of ^{11}C-PIB retention has been related to the distribution of cerebral atrophy measured with MRI and changes in the default network [124]. In contrast another study suggested that the

cerebral atrophy is more focal compared to changes in CMRglc than the more widespread distribution of amyloid deposition [69]. Further studies are needed to relate different pathological and functional parameters in AD and establish their regional and temporal relationships.

It seems that the pathological changes in the brain with amyloid deposition closely follow the biomarkers in CSF. We have previously shown that the amyloid deposition is quite stable in AD patients [187] and the same thing has been shown for CSF biomarkers [50], suggesting that these parameters reach a plateau although the functional changes progressively decline. These results suggest that said pathological markers might be suitable for earlier diagnosis of AD and useful to evaluate new treatment strategies focusing on increasing amyloid clearance or reducing Aβ production.

The findings in this study indicate that there might be differences in time course between the pathological and functional changes. Amyloid deposition in the brain seems to be an early event in the course of the disease and is related to concentrations of CSF biomarkers. Notably the functional changes with decrease in CMRglc seem to start somewhat later and show a strong correlation with memory performance. Convergences of these pathological and functional changes are found in the posterior cingulum and parietotemporal regions of the brain. These areas are known to be involved in successful memory retrieval and regional default activity together with AD related changes including amyloid deposition and decrease in CMRglc predisposes to disease progression and memory impairment. Prospective studies of MCI patients and persons with nonsymptomatic familial forms of AD are needed, and these subjects need to be followed longitudinally to elucidate the interrelationships between pathological and functional changes in the development of AD.

5 CONCLUSIONS AND FUTURE ASPECTS

The aim of this thesis was to evaluate the use of positron emission tomography as a tool for early detection of AD pathology and the progress of the disease.

Our findings in patients with mild cognitive impairment showed that about half of the patients had high ^{11}C-PIB retention and all seven patients that later converted to AD had high ^{11}C-PIB retention. The amyloid deposition seems to be an early event in the development of AD preceding decrease in glucose metabolism and cognitive deficits (Figure 13).

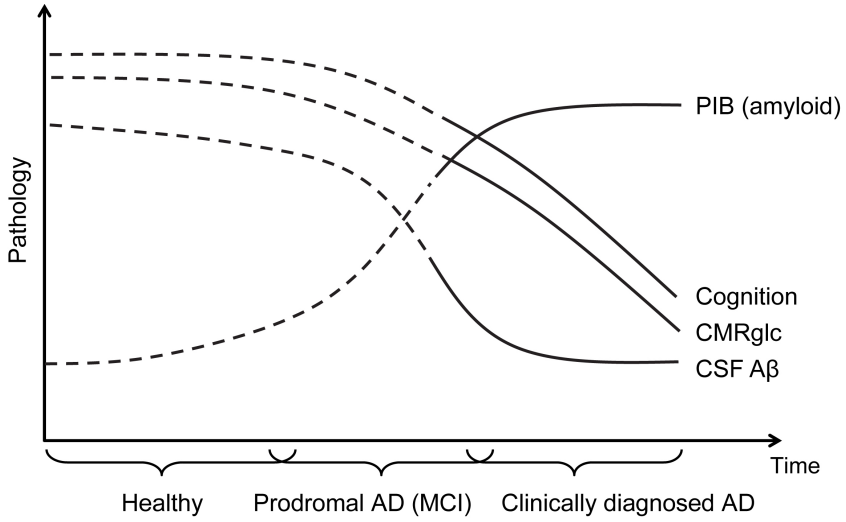

Figure 13. Hypothesis of the time-line for pathological changes in the brain and CSF as well as functional changes in the brain and cognitive measurements.

These findings suggest that ^{11}C-PIB PET might be a good method together with neuropsychological investigations and measurement of biomarkers in CSF for early detection of patients with very high risk of developing AD. We have been able to show that amyloid depositions measured with ^{11}C-PIB PET is, at a group level, quite stable over a period of up to 2.5 years. We could still witness a progression of the disease with decline in CMRglc, measured with ^{18}F-FDG PET, and cognitive status. It was evident that the pathological amyloid deposition in brain, measured with ^{11}C-PIB PET, was correlated with pathological biomarkers measured in CSF (Figure 12B). The correlation was significant when studying the entire material of AD and MCI patients together and the MCI patients separately but not for AD alone. This might be due to the need of variance in the material to find correlation and at later stages of the disease all patients have high amyloid load in the brain, low $A\beta_{1\text{-}42}$ or high tTau or pTau in CSF. The functional status of the brain measured with ^{18}F-FDG PET is closely related to the progression of the disease measured by neuropsychological tests, especially episodic memory. The ^{11}C-PIB retention is to a less extent related to severity of the disease but shows a correlation to disease state in the total material with both AD and MCI patients. In the third study we could also see that the dynamic ^{11}C-PIB PET scan can give both pathological information of amyloid load and functional estimates of CBF.

One fundamental question is what pathological changes are the first to trigger neuronal damage that inevitably leads to the progressive cognitive decline. The bulk of AD research has so far led to development of the amyloid cascade theory as the leading hypotheses for AD etiology [6]. The Aβ pathology spreads from orbitofrontal and temporal brain regions to the rest of frontal cortex and the parietal lobe and to finally include most of the neocortex [191]. The same topographical and temporal distribution of amyloid pathology was recently described using ^{11}C-PIB PET [131, 192]. In line with these findings it was recently argued by Buckner and colleagues that activity in the default network, including parietotemporal brain regions and posterior cingulum, in early adulthood might be involved in susceptibility to amyloid deposition and development of AD [124]. These assumptions were based on clear topographical similarities between the amyloid deposition and disturbances in the default network arguing a relationship between these processes, one that needs further research to establish. These topographical similarities of amyloid depositions and activity changes in the neuronal network were also found in a recent study by Nelissen et al [179]. It is intriguing to witness how these functional and pathological changes are intertwined in the development and progression of the disease. Early decline in cerebral glucose metabolism has been shown in MCI patients and seems to predict further conversion to AD [65]. Changes in glucose utilization have even been found in nonsymptomatic subjects genetically at risk to develop AD being ApoE-ε4 homozygotes [193]. Based on the findings presented in this thesis I would like to argue that amyloid deposition is the earliest event in the AD etiology preceding neuronal death and changes in glucose metabolism in the brain networks. In my mind these findings of early changes in glucose metabolism and neuronal network activity in AD patients and nonsymptomatic ApoE-ε4 carriers are involved in the susceptibility to pathological changes and time of disease onset and rate of progression. This theory is supported by the fact that early amyloid deposition is evident in MCI patients without any clear regional decrease in glucose metabolism. Correspondingly, there was no correlation between amyloid depositions in MCI patients alone but in AD patients in parietal cortex, a brain region known to show progressive deterioration of CMRglc [61]. A possible hypothesis is that these patients are capable of coping with the pathological strain on the brain with sustained CMRglc, CBF and a relatively intact cognitive status. This notion was recently supported by research showing evidence of cognitive reserve with high-educated AD patients showing more severe pathology and impaired brain function with high amyloid load measured with ^{11}C-PIB PET and lower CMRglc measured with ^{18}F-FDG PET than equally demented low-educated AD patients [186]. The hypothesis of cognitive reserve suggests that a more efficient neuronal network may compensate for disruption caused by pathological processes [194], something that might explain the fact that we witnessed stable MCI patient with very high ^{11}C-PIB retention but quite intact cognitive performance CMRglc. The order of pathological changes and functional impairment discussed in this thesis is in line with the amyloid hypotheses stating that amyloid toxicity and pathology precedes tau pathology, neuronal death, alterations in neurotransmitter systems, grey matter atrophy, memory impairment and cognitive decline [13].

Studies with ^{11}C-PIB PET are performed in similar ways but still show some methodological differences. It is therefore important to discuss these dissimilarities and how they might impact the results presented in this thesis and other publications. Analysis of different pharmacological models for calculation of quantitative or semi-

quantitative data for ^{11}C-PIB PET has been done thoroughly [115]. Data produced by either graphical models and simplified late scan ratio calculation resulted in reliable data for discrimination between AD and HC, although the more advanced methods gave less test/retest variability [115]. It was still evident that a late scan ratio between 40-60 minutes of the ^{11}C-PIB PET scan was sufficient to produce reliable data of ^{11}C-PIB making it possible to shorten the time in the camera that would give relief for the patient. This method of data quantification was used in this thesis and several other research studies with ^{11}C-PIB PET showing that it is a robust method [117, 119, 121]. Different methodological tools for image analysis have been used in amyloid imaging including the whole span from visual assessment [134], ROIs drawn by hand [100], automatic ROIs [117, 195] and voxel based analysis [117, 196]. All methodological modalities producing the same clear differentiation between AD patients and healthy controls strengthening the notion that ^{11}C-PIB PET is a stable method independent of the quantification and evaluation method used. We and others have shown that it is possible to spatially normalize the late scan ratio ^{11}C-PIB images using images of the early frames of the ^{11}C-PIB PET scan [121]. Nevertheless the use of structural imaging data for spatial normalization increases the accuracy of the statistics and would be needed to take advantage of new PET cameras with higher resolution and give the possibility to study the amyloid deposition in areas that today are hard to exactly locate as the hippocampus and entorhinal cortex. The use of refined methods for quantification and image analysis, including automatic ROI programs and voxel based methods without any *a priori* hypothesis of affected brain areas will be of great importance in diagnostics, evaluation of disease processes and new treatment strategies. One recent attempt to find a diagnostic approach used SPM to extract voxels of interest that were then used to separate AD patients and healthy controls with good result [197]. Prospective studies are needed to evaluate the true diagnostic value of this and similar approaches.

The development of means to study AD pathology *in vivo* will be of great use in research and evaluation of new treatment strategies targeting amyloid and tau. Only one study has so far used amyloid imaging with ^{11}C-PIB PET to study treatment effects with a newly developed drug shown to influence Aβ production, namely phenserine [166]. The study also involved evaluation of functional changes including CMRglc measured with ^{18}F-FDG PET and neuropsychological tests. Additional studies are needed to elucidate the effect of treatment strategies targeting amyloid production and clearance to further evaluate the reliability of the amyloid cascade theory.

Future aspects for amyloid imaging would involve larger cohorts in longitudinal studies of MCI patients to elucidate what predictive value amyloid imaging have in foreseeing conversion to AD. We are the only ones that have tried to relate the amyloid deposition, measured with, ^{11}C-PIB PET, to conversion from MCI to AD but larger studies are needed. It is also important to study familial forms of AD and investigate when the amyloid deposition starts and how that is related to functional changes and cognitive decline.

The final conclusion is that ^{11}C-PIB PET shows great potential as a tool for earlier diagnosis, studying the progression of disease and evaluation of future treatment strategies; and thereby being an important part in the research that will prove or discard the amyloid cascade theory.

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