From the Division of Alzheimer Neurobiology Center
Department of Neurobiology, Care Sciences and Society
Karolinska Institutet, Stockholm, Sweden

A MULTI-TRACER PET APPROACH TO STUDY EARLY-ONSET FAMILIAL AND SPORADIC ALZHEIMER'S DISEASE

Michael Schöll

Karolinska Institutet

Stockholm 2011

The cover shows three-dimensional renderings of PET scans in the same individual using the three tracers employed in this thesis (from above: ^{11}C-DED, ^{18}F-FDG, ^{11}C-PIB).

Published by Karolinska Institutet. Printed by Larserics Digital Print AB

ISBN 978-91-7457-458-6

Dedicated to my family, the patients and their relatives

"If anyone supposes that he really knows anything,
he has not yet learned as he ought to know." (1 Cor 8:2)

Abstract

Cumulated scientific evidence suggests that the pathology causing Alzheimer's disease (AD) occurs many years or even decades before memory impairment and other clinical symptoms arise. Tangible and detailed knowledge about different pathological processes, their interactions, and time course is therefore of the essence both for the development of potentially successful treatments and a reliable early diagnosis of this relentless disorder. The past decade has thus seen an explosion in research on biomarkers that could provide *in vivo* evidence for these pathological processes, involving β-amyloid (Aβ) production and aggregation into plaques, neurofibrillary tangle formation, neuroinflammation, and eventually neurodegeneration.

The rare form of dominantly-inherited early-onset familial AD (eoFAD), with almost complete mutation penetrance and defined age of disease onset, has been proposed as a model to study the very early disease mechanisms that are also supposed to underlie the common sporadic form (sAD). However, more than 200 mutations in three different genes (*PSEN1 and 2, APP)* have been identified as causing eoFAD, some of which have been shown to differ substantially from others.

This work employed multi-tracer positron emission tomography (PET), using the tracers 2-[^{18}F]-fluoro-2-deoxy-D-glucose (FDG), *N*-methyl-[^{11}C] 2-(4'-methylaminophenyl)-6-hydroxy-benzothiazole (PIB), and [^{11}C]-L-deuterium-deprenyl (DED) to explore the characteristics, time course and interrelationship of cerebral glucose metabolism, fibrillar Aβ burden, and astrocyte activation (astrocytosis) at different pre-symptomatic and symptomatic disease stages of eoFAD and sAD, in relationship to cognition, other AD biomarkers, and/or *post-mortem* pathology.

Thalamic hypometabolism in *PSEN1* eoFAD mutation carriers was demonstrated in this thesis nearly 20 years before they were expected to develop clinical symptoms. The pattern of hypometabolism studied in several mutation carriers spread subsequently to regions that are also typically affected in sAD, correlating well with cognitive decline at symptomatic disease stages. Regional hypometabolism was furthermore found to correlate with typical AD pathology, namely neuritic Aβ plaques at *post-mortem* examination, suggesting that FDG PET is an excellent marker of disease progression from early pre-symptomatic stages to terminal disease.

One particular eoFAD mutation, the Arctic *APP* mutation, has been reported to modify amyloid processing in a way that obviates the formation of fibrillar Aβ, the form of Aβ most prone to aggregate into neuritic plaques. In contrast to carriers of other eoFAD mutations and sAD patients, we found that carriers of the Arctic *APP* mutation showed no cortical PIB PET retention as a measure of fibrillar Aβ load, while Aβ and tau in cerebral spinal fluid and glucose metabolism, and in advanced disease also medial temporal lobe atrophy as measured by magnetic resonance imaging and cognition were clearly pathological and typical of AD. The findings imply that clinical AD can be caused by forms of Aβ, supposedly oligomeric or protofibrillar, which cannot be detected by PIB PET.

Very little is still known from *in vivo* studies about when and where in the brain neuroinflammation occurs in AD. Here, it could be shown that DED binding as a measure of astrocytosis was elevated in prodromal AD patients, whereas binding levels in AD were comparable to those in controls. PIB PET retention was increased and glucose metabolism decreased in both groups and there was no regional relationship between the three tracers, indicating that astrocytosis is an early phenomenon in AD that follows a different spatial

and temporal pattern than Aβ plaque deposition and impaired synaptic activity as measured by glucose metabolism.

Multi-tracer PET is in this work proven to provide novel insights in eoFAD and sAD pathogenesis with processes such as astrocytosis and the potential role of different Aβ species. This knowledge is of significance for the understanding of disease mechanisms as well as the comparability of the purely genetic and the sporadic form of AD.

Sammanfattning på svenska

Forskning har visat att patologin som orsakar Alzheimers sjukdom (AD) inträffar många år innan de första kognitiva symptomen uppstår. Det är därför avgörande att öka förståelsen om den patologiska processen för att lyckas utveckla framgångsrik behandling och för att kunna ställa en tidig tillförlitlig diagnos. Under det gångna årtiondet har många nya biomarkörer upptäckts som påvisar tidiga patologiska förändringar, som bland annat beta amyloid (Aβ) produktion och aggregering till plack, formation av neurofibrillära nystan, neuroinflammation och slutligen neurodegenereration.

AD kan delas upp i den sporadiska och den familjära formen beroende på huruvida orsaken till sjukdomen är genetisk betingad eller inte. Den ovanliga dominant-nedärvda familjära formen av AD (eoFAD) som medför tidigt insjuknade, har föreslagits som en bra modell för att studera tidiga sjukdomsmekanismer som kan ligga bakom även sporadisk AD (sAD). Mer än 200 mutationer i tre olika gener, presenilin-1 (*PSEN1*), presenilin-2 (*PSEN2*) och amyloid precursor protein (*APP*), orsakar eoFAD som i vissa fall skiljer sig väsentligt åt i bland annat sjukdomsförlopp.

I denna avhandling har vi använt oss av så kallad "multi-tracer" positron emission tomografi (PET), en molekylär imaging metod som kombinerar olika spårämnen för att kunna studera samband mellan fysiologiska och patologiska processer på molykär nivå. Vi använde spårämnena 2-[^{18}F]-fluoro-2-deoxy-D-glucose (FDG), *N*-methyl-[^{11}C] 2-(4'-methylaminophenyl)-6-hydroxy-benzothiazole (PIB) och [^{11}C]-L-deuterium-deprenyl (DED) för att studera glukosmetabolism, amyloida plack och astrocyt aktivering. Vi undersökte dessa processer för att utforska förhållandet mellan dem innan sjukdomen brutit ut och då symptom har börjat visa sig och diagnos har ställts i både eoFAD och sAD.

I den första studien har vi studerat bärare av en PSEN1 mutation och observerat nedsatt glukosmetabolism i en specifik region, thalamus, nästan 20 år innan förväntad ålder för insjuknande. Hypometabolismen spred sig sen till områden påverkade även i sAD och korrelerade väl med den kognitiva försämringen. Den regionala hypometabolismen upptäcktes även korrelera med antalet Aβ plack i samma område efter undersökning av dessa region efter patientens död. Detta visar att FDG PET är en bra markör för att följa sjukdomsutvecklingen från långt innan symptom visar sig ända till senaste sjukdomsskeden.

Denna avhandling har vidare studerat bärare av en annan eoFAD mutation i APP genen kallad den Arktiska mutationen. Denna mutation har rapporterats modifiera Aβ processningen så att ingen fibrillär Aβ med en viss struktur bildas vilket förhindrar aggregering till neuritiska plack. I motsats till bärare av andra eoFAD mutationer och i sporadisk AD, har vi upptäckt att bärare av den Arktiska mutationen inte visar något upptag av PIB PET i hjärnbarken samtidigt som andra biomarkörer såsom Aβ och tau i cerebrospinal vätskan, glukosmetabolism och atrofi i vissa delar av hjärnan visade typiska AD mönster. Våra upptäckter visar på att AD kan orsakas av inte bara fibrillär Aβ, utan även av andra former, sannolikt oligomera eller protofibrillära, som inte kan detekteras av PIB PET.

I den tredje studien har vi studerat neuroinflammation som fortfarande är tämligen outforskat *in vivo.* Vi använde oss av DED PET för att mäta astrocyt aktivering och fann förhöjd detektion hos patienter med mild kognitiv svikt som med all sannolikhet kommer att utveckla AD vilket förespråkar att astrocyt aktivering skulle vara ett tidigt fenomen i AD.

Sammanfattningsvis, PET tillhandahåller inblick i den patologiska processen i både eoFAD och sAD som är av stor vikt för att öka förståelsen av bakomliggande sjukdomsmekanismer och för att följa patologiska förändringar från långt före till efter insjuknande.

Abbreviations & Acronyms

Aβ	β-amyloid
ACh	Acetylcholine
AChEI	Acetylcholinesterase inhibitor
AD	Alzheimer's disease
ADAS-cog	Alzheimer's Disease Assessment Scale - cognitive subscale
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
CAA	Cerebral amyloid angiopathy
CMRglc	Cerebral metabolic rate of glucose
CNS	Central nervous system
CSF	Cerebrospinal fluid
DED	[^{11}C]-L-deuterium-deprenyl
DLB	Dementia with Lewy-bodies
eoAD	Early-onset Alzheimer's disease
eoFAD	Early-onset familial Alzheimer's disease
FAD	Familial Alzheimer's disease
FDG	2-[^{18}F]-fluoro-2-deoxy-D-glucose
fMRI	Functional magnetic resonance imaging
FTD	Frontotemporal dementia
loAD	Late-onset Alzheimer's disease
MCI	Mild cognitive impairment
MMSE	Mini mental state examination
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NFTs	Neurofibrillary tangles
NPs	Neuritic plaques
PDD	Parkinson's disease dementia
PET	Positron emission tomography
PIB	*N*-methyl-[^{11}C] 2-(4'-methylaminophenyl)-6-hydroxy-benzothiazole (Pittsburgh Compound B)
PSEN	Presenilin
sAD	Sporadic Alzheimer's disease
SPECT	Single photon emission computed tomography
VAD	Vascular dementia

Publications

This thesis is based on the following papers:

Paper I

Schöll M, Almkvist O, Axelman K, Stefanova E, Wall A, Westman E, Långström B, Lannfelt L, Graff C, Nordberg A.

Glucose metabolism and PIB binding in carriers of a His163Tyr presenilin 1 mutation.

Neurobiol Aging. 2011 Aug;32(8):1388-99. Epub 2009 Sep 30.

Paper II

Schöll M, Almkvist O, Bogdanovic N, Wall A, Långström B, Viitanen M, Nordberg A.

Time course of glucose metabolism in relation to cognitive performance and postmortem neuropathology in Met146Val PSEN1 mutation carriers.

J Alzheimers Dis. 2011 Jan 1;24(3):495-506.

Paper III

Schöll M, Wall A, Thordardottir S, Ferreira D, Bogdanovic N, Rasmuson S, Byström A, Långström B, Almkvist O, Graff C, Nordberg A.

Low PIB PET retention in presence of pathological CSF biomarkers in Arctic APP mutation carriers

Submitted manuscript

Paper IV

Carter SF*, **Schöll M***, Almkvist O, Wall A, Engler H, Långström B, Nordberg A.

**contributed equally*

Evidence for astrocytosis in prodromal Alzheimer's disease provided by ^{11}C-Deuterium-L-Deprenyl - A multi-tracer PET paradigm combining ^{11}C-PIB and ^{18}F-FDG

Accepted for publication in the Journal of Nuclear Medicine

Contents

Introduction

Alzheimer's disease

When in 1901 the German psychiatrist and neuropathologist Alois Alzheimer first met Auguste Deter, a 51-year-old woman who described her strange state of delusion and forgetfulness with the words "I have lost myself", he could hardly have imagined that this would be the beginning of more than a hundred years of effort and struggle against a relentless disease striking millions all over the globe. In his publication "Über eine eigenartige Erkrankung der Hirnrinde" [1], which alluded to a *post-mortem* examination of Auguste Deter's brain, Alzheimer later described the still valid major hallmarks of what thereafter would be called Alzheimer's disease (AD).

AD is the most common form among a group of symptoms described as dementia, accounting for between 50 and over 70% of all cases [2]. Apart from AD, this syndrome includes disorders such as frontotemporal dementia (FTD), cerebrovascular dementia (VAD), dementia with Lewy-bodies (DLB), and Parkinson's disease dementia (PDD). With an estimated 29 million patients suffering from dementia worldwide in 2005, it ranks among the major causes of death and constitutes a considerable economic burden which has been calculated to approximate US$ 315 billion [3]. The prevalence of dementia is closely related to age, with a rate of only 1% in 60- to 64-year-olds, but up to 33% in individuals aged 85 years or older [4]. Given the global increase in life expectancy, the prevalence of AD has also been predicted to rise globally to 80 million patients within the next 40 years [5].

AD is a lethal, progressive neurodegenerative disorder that leads to a clinical picture of gradually deteriorating memory with subsequent appearance of other cognitive, behavioural, and neuropsychiatric changes that impair social interactions and activities of daily living, until the afflicted person is completely dependent on caregivers [6]. The inexorable disease progression thereby puts immense pressure on AD patients' families and their social environment.

Typically, individuals with AD can be subdivided into cases of sporadic AD (sAD) (sporadic: "occurring in irregular or random instances" [7]), and familial AD (FAD), with sAD accounting for the vast majority of all AD cases.

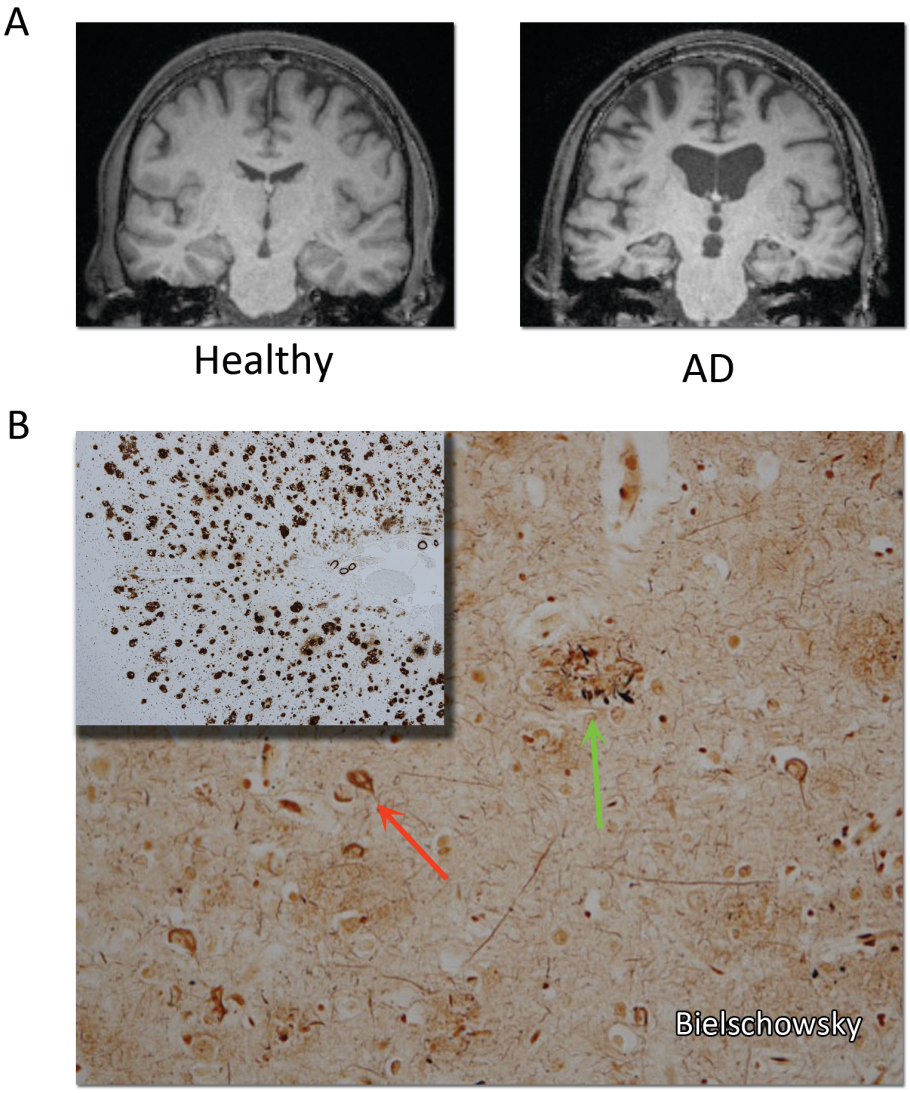


Figure 1. Characteristic AD pathology. (A) Atrophy of the brain as visualised by magnetic resonance imaging. (B) Neuritic plaques (green arrow) and neurofibrillary tangles (red arrow) as seen under the microscope (Bielschowsky staining). The small window shows intense cortical plaque pathology (Microscopy images courtesy of Dr Nennesmo, Karolinska University Hospital Huddinge).

A further subdivision can be made into those who develop symptoms under the age of 65, early-onset AD (eoAD), and the far more common form that develops at ages over 65, late-onset AD (loAD). Only about 1% of all AD patients belong to the specific subgroup of early-onset familial AD (eoFAD) which is caused by autosomal-dominant gene mutations [8]. The terms FAD and eoAD may describe eoFAD, but may also include sAD cases with a family history of the disease or AD associated with non-dominant genetic factors such as the apolipoprotein (APOE) ε4 allele.

Pathology and pathogenesis

When Dr Alzheimer examined Auguste Deter's autopsied brain, he found substantial shrinkage (atrophy) of the brain macroscopically and, under the microscope, two of the most typical hallmarks of AD: extracellular senile or neuritic plaques and intracellular neurofibrillary tangles (NFTs) *(Figure 1)* [1]. With increasing scientific knowledge, however, many more pathological features of AD such as neuroinflammatory processes have been discovered and described.

β-Amyloid

A crucial finding was made in the 1980s, when a fibrillar, insoluble form of the β-amyloid (Aβ) protein was identified as the main component of the neuritic plaque cores [9, 10]. Aβ was originally believed to be exclusively formed under pathological conditions. At the beginning if the 1990s, however, it was reported to be derived under physiological conditions by proteolytic cleavage from its precursor, amyloid precursor protein (APP) [11, 12]. APP is a ubiquitously expressed, type 1 integral membrane protein that exists predominantly as three different isoforms: APP695, APP751, and APP770, whereof APP695 is the most abundant isoform in the brain [13]. Three enzymes were found to account for APP proteolysis and were subsequently denominated α-, β-, and γ-secretase. These cleave APP in either of two exclusive ways, a non-amyloidogenic (or α-secretase) and an amyloidogenic (or β-secretase) pathway *(Figure 2)*.

The non-amyloidogenic pathway first involves α-secretase cleavage of APP within the Aβ domain, resulting in the soluble α-APPs fragment, followed by γ-secretase cleavage of the remaining C-terminal fragment (α-CTF or C83), which releases a peptide called p3 and the APP intracellular C-terminal domain (AICD) [14-17]. The amyloidogenic pathway, however, results in release of Aβ peptides of different lengths [18, 19]. First, APP is cleaved by β-secretase (BACE1) at the β-site (N-terminal position 1), forming the soluble fragment β-APPs [20]. The remaining β-CTF (C99) is then cleaved by γ-secretase at different sites, releasing Aβ peptides of 39–43 amino acids in length. Under physiological conditions, $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ form the majority of peptides, with $A\beta_{1\text{-}40}$ being by far the most abundant [9, 21]. The hydrophobic, fibrillogenic $A\beta_{1\text{-}42}$ species aggregates more readily than other species into soluble oligomers, protofibrils, and eventually insoluble fibrils characterised by a β-pleated sheet conformation, which stains with Congo

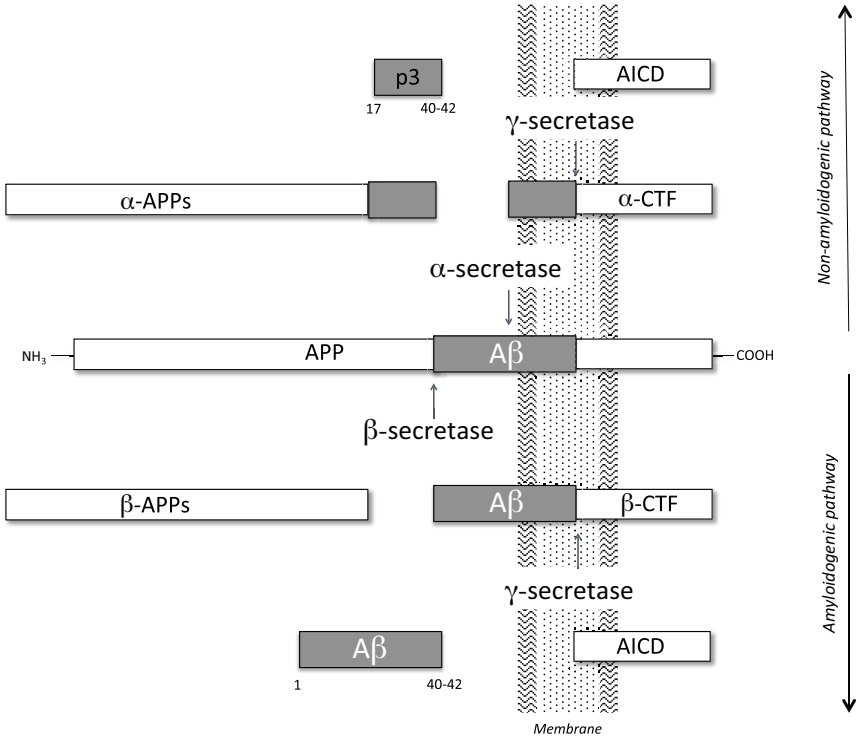


Figure 2. Amyloidogenic and non-amyloidogenic metabolism of APP.

red and Thioflavin-S, eventually forming the AD-typical neuritic plaques [21, 22]. Besides A$\beta_{1\text{-}40/42}$, N-terminal truncated Aβ peptides such as A$\beta_{3\text{-}40/42}$, A$\beta_{11\text{-}40/42}$ and A$\beta_{17\text{-}40/42}$, and several other proteins accumulate within these plaques. When Aβ is deposited in non-fibrillar (non-β-sheet) conformations, it forms so-called diffuse plaques. Aggregates of Aβ can furthermore be found in blood vessel walls in many AD cases, a phenomenon called cerebral amyloid angiopathy (CAA) [23].

Physiologically, Aβ can be degraded by numerous enzymes. The most extensively studied are insulin-degrading enzyme and neprilysin – their levels have been demonstrated to be reduced in AD [24]. Likewise, clearance of Aβ from the central nervous system has been shown to be decreased in AD patients [25].

The majority of Aβ plaques are found in the grey matter of the brain. Progression of amyloid plaque pathology generally proceeds in a particular manner, and depending on the brain regions affected this can be roughly classified into three widely recognised stages (A-C), as first proposed by Braak and Braak in 1991 [26, 27], or somewhat more precisely into five phases, as proposed by Thal, Braak, and colleagues in 2002 [28]. At Braak stage A, low densities of Aβ deposits are found in the basal neocortex of the temporal lobe. Stage

B shows an increase in Aβ deposits in almost all isocortical association areas and only the primary sensory areas and primary motor field remain almost devoid of deposits. There is mild involvement of the hippocampal formation, and Aβ may be found in the entorhinal cortex. Stage C is mainly characterised by virtually all isocortical areas being affected [27].

The finding that Aβ deposition follows a hierarchical pattern in the medial temporal lobe (MTL) [29] led to a thorough examination of the hierarchical pattern of Aβ deposition in the entire brain. Phase 1 was described as pure neocortical Aβ deposits, while Phase 2 involves additional allocortical brain regions. Phase 3 involves Aβ deposits in diencephalic nuclei, the putamen, the caudate nucleus, and several cholinergic nuclei of the basal forebrain; brainstem nuclei are first affected in Phase 4. In Phase 5, additional brain stem nuclei and the cerebellum are finally affected [28].

Aβ plaque burden and cognitive impairment have been reported to correlate only weakly or not at all [30-32]. Likewise, neither clinical disease nor specific functional impairment has been shown to correlate well with Aβ deposition [33, 34].

The seminal discovery that mutations in the gene encoding APP could cause AD (see also "Genetics"), led to articulation of the amyloid cascade hypothesis *(Figure 3)* by Hardy and Higgins in 1992, which posits that an imbalance between deposition and clearance of Aβ in the brain is the central event in AD pathogenesis, initiating other pathological events until the manifestation of AD dementia [35]. The hypothesis has been massively criticised [36] and been subject to many modifications, but it is undoubtedly the predominant hypothesis in AD research and has been agenda-setting in drug development. In its beginnings, it was believed that the fibrillar, insoluble Aβ plaques were the cause of the disease [35]. Since then, the most substantial revision has been the inclusion of a concept that states that non-fibrillar (or pre-fibrillar) soluble Aβ oligomers are the most toxic species [37, 38]. Early evidence that these forms might play a role in memory impairment came from human brain tissue analyses that showed significant correlations between cortical levels of soluble Aβ and cognitive impairment as well as with the extent of synaptic loss [39, 40]. Numerous studies *in vitro* and *in vivo* in transgenic animal models have in fact confirmed that these soluble oligomeric Aβ or protofibrillar intermediate species and synthetically assembled Aβ-derived diffusible ligands (ADDLs) decrease or block long-term potentiation (LTP), a mechanism considered to underlie aspects of learning and memory, and to cause neuronal loss, as well as induction of an inflammatory response [41-45].

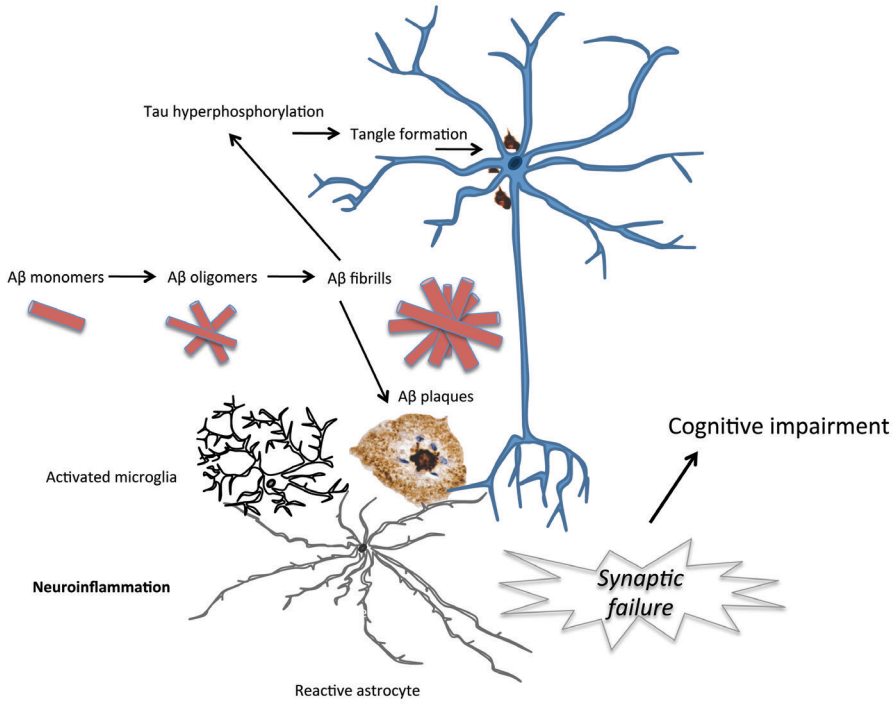


Figure 3. According to the amyloid cascade hypothesis, accumulation of Aβ is the central event in AD pathogenesis, leading to NFT formation, triggering inflammatory processes, and causing synaptic dysfunction and neurodegeneration until cognitive deficits result in dementia.

A differing pattern of oligomer species has been identified in a recent study of human *post-mortem* brain tissue between eoAD and loAD [46].

Of note is the fact that the term soluble Aβ is an operational definition, including all forms of Aβ that remain in solution following high-speed centrifugation of brain extracts. These have exclusively been studied in artificial environments and have not yet been shown to occur *in vivo*.

Tau

Shortly after Aβ was discovered to be the main component of neuritic plaques, neurofibrillary tangles (NFTs) were shown to consist of the hyperphosphorylated form of a neuronal microtubule-associated protein called tau [47]. Tau is mainly found in axons but can also occur at much lower levels in dendrites, exerting functions that are not completely understood [48-50]. The best-established biological functions are the stabilisation of the microtubule network and the regulation of axonal transport [51, 52].

In AD (and other so-called tauopathies), tau pathology is characterised by the somatodendritic accumulation of hyperphosphorylated tau, which eventually aggregates

into NFTs [53]. The phosphorylation of tau depends on the balance between different kinases and phosphatases [54]. Disruption of this balance as seen in AD leads to the sequestration of tau, which aggregates into insoluble tangles, causing microtubule disassembly and subsequent impairment of axonal transport, and synaptic and neuronal integrity [54].

If tau hyperphosphorylation and accumulation are a cause or a consequence of AD is still an unsolved issue. According to the amyloid cascade hypothesis, tau pathology is driven by Aβ formation (see "Pathology: β-Amyloid"). A recent publication concerns three possible modes of interaction between Aβ and tau pathology, mainly based on findings in transgenic animal models. These suggest that (A) Aβ either actually triggers tau pathology, (B) tau mediates the toxic effects of Aβ, or that (C) Aβ and tau have synergistic toxic effects on different components of the same system, exemplified by their effects on mitochondrial respiration [52].

The deposition of AD-related NFTs pathology follows a certain hierarchical pattern, which differs fundamentally from Aβ deposition. It was originally categorised by Braak and Braak into six stages in 1991 [26]. To enhance accuracy of the staging and meet the demands of clinical routine, these stages were re-evaluated using much thinner sections and improved staining [55]. The first stage involves lesions in the transentorhinal regions; in stage II these lesions extend into the entorhinal region, and in stage III further into neocortical areas of the fusiform and lingual gyri. From there, tau pathology spreads more widely into neocortical association areas (stage IV), frontal, superolateral, and occipital neocortex (stage V), and finally most areas of the neocortex are affected (stage IV). In the final stages (V and VI), a 91% agreement on the diagnosis of AD was reached within the BrainNet Europe Consortium, while milder lesions (stages I and II) led to a more diverse judgement [56].

The patterns of Aβ plaques and tau pathology differ not only spatially but also temporally. Tau pathology has consistently been reported to precede Aβ deposition [27, 57] and to correlate far better with cognitive impairment and thus clinical disease [32, 58]. Interestingly, pretangle tau material has been found in the majority of brains from a group of individuals aged 4 to 29 in the absence of Aβ protein deposition [59]. Here, pathology started in subcortical, non-thalamic nuclei in most of the examined cases, which further led to the hypothesis that tau pathology may spread from there via neuron-to-neuron propagation [60].

Neuroinflammation

Inflammation is the immune response of an organism towards harmful agents or events. An immune response is carried out by specialised cells that under physiological conditions may exert a wide variety of other functions. Inflammatory processes are crucial and necessary but can turn harmful e.g. in their chronic stages. Neuroinflammation, a localised inflammatory response in the CNS, is a prominent feature of AD but as with many aspects of AD pathology, we still do not know whether inflammatory processes represent a driving force for or a consequence of other pathology. Brain resident cells, namely microglia, astrocytes, and neurons, mediate neuroinflammatory processes. Early findings of activated microglia in neuritic plaques from AD brains led to the assumption of an inflammatory component being involved in AD pathogenesis [61]. This idea was confirmed by the fact that treatment with non-steroidal anti-inflammatory drugs (NSAIDs) had been shown to lower the risk of developing AD [62, 63].

Microglia are cells related to the mononuclear phagocytic system, expressing proteins typical of phagocytic cells such as the major histocompatibility complex (MHC) and complement components [64]. When resting, they appear in a typical ramified shape, extending their processes into the surroundings in order to detect abnormalities [65]. On activation, microglia turn into more motile amoebic cells [66], releasing inflammatory mediators including neurotoxic cytokines such as interleukins and tumour necrosis factor α (TNF-α) [67]. As mentioned above, microglia can often be found in or around neuritic, not diffuse plaques [68]. Aβ can trigger activation of microglia, which in a reciprocal manner, mediated by interleukins, increases the production of APP and its secreted fragments [69, 70]. This vicious circle might contribute to the chronic neuroinflammation in AD.

Astrocytes are the most abundant and diverse cell type in the brain. They appear in manifold morphological shapes and are organised in a huge network that involves connections of a single astrocyte with hundreds of neurons and thousands of synapses, as well as gap junctions between individual astrocytes [71]. Amongst the large variety of functions carried out by astroglial cells, maintenance and support of neuronal functions and physiology, formation of the blood-brain barrier, and clearance of neurotransmitters from the synaptic cleft could be considered most notable. Their role in brain energy homeostasis and metabolism is of particular interest as regards molecular imaging of cerebral glucose metabolism by means of positron emission tomography [72, 73] (see

"PET in AD: Glucose metabolism"). Close neuronal-astrocytic metabolic coupling, represented by astrocytic provision of lactate to neurons, has also been shown to be of importance with regard to memory formation [74], a process profoundly impaired in AD, and it affects neuronal integrity in the presence of Aβ aggregates [75].

AD brains show prominent astrogliosis, predominantly around Aβ plaques [76-78]. Aβ has been found in astrocytes surrounding neuritic plaques, recently also in the form of Aβ protofibrils [79-81]. Of note is the fact that astrocytes have been shown to participate both in the formation of Aβ plaques and in Aβ clearance [82-84]. The fact that (activated) astrocytes express β-secretase underlines their potentially important role in Aβ pathogenesis [85]. Other aspects of AD pathology might also be partly mediated by astrocytes. Parallel to hypertrophic astrogliosis close to neuritic plaques, astroglial atrophy distant from the plaques has been found in transgenic mice, leading to impaired astrocytic support for synaptic processes [84]. The degeneration of astrocytes hereby preceded the formation of both plaque and tangle pathology.

Genetics

Early-onset familial AD

Genetic predisposition is a well-known risk factor and cause of AD, as family histories involving early age at onset (younger than 65 years) and a clear autosomal dominant pattern of inheritance with virtually 100% penetrance were recognised as early as in the 1930s [86]. It has been speculated that Auguste Deter, the first reported AD case, was part of a Volga-German family harbouring an autosomal dominant AD mutation and thus suffered from eoFAD [87]. Mutations in three genes — the β-amyloid precursor protein gene (*APP*) on chromosome 21 [88], the presenilin 1 gene (*PSEN1*) on chromosome 14 [89], and the presenilin 2 gene (*PSEN2*) on chromosome 1 [90, 91] — have been found to be direct causes of eoFAD. *PSEN1* mutations have been estimated to account for 80 - 90% of all eoFAD cases. The phenotypes associated with these mutations show early onset of the disease with a specific age at onset for a given pedigree. Several families harbouring eoFAD mutations have been discovered in Sweden, these are listed in *Table 1*.

Mutation	Mean expected age of disease onset (y)
***PSEN1* Ile143Thr**	34
***PSEN1* Met146Val**	37.7
***PSEN1* His163Tyr**	54.2
***PSEN1* Arg269His**	55.1
***APP* KM670/671NL (Swedish *APP*)**	55
***APP* Glu693Gly (Arctic *APP*)**	59.7

Table 1. EoFAD mutations identified in Swedish pedigrees.

APP

Since the Aβ peptide was isolated from plaque cores from patients with AD and from patients with Down's syndrome (trisomy of chromosome 21) [92], the search for a gene encoding Aβ was initially focused on chromosome 21. In 1991, Hardy and colleagues identified a missense (point) mutation in the APP gene (*APP*) in kindred with linkage to chromosome 21 markers - the link between AD, Aβ and genetics was established [88]. Following this initial discovery, 31 dominant pathogenic *APP* missense mutations and *APP* locus duplications have been found to date, accounting for an estimated 16% of all eoFAD cases [www.molgen.ua.ac.be/admutations, [93]. In addition, two recessive *APP* mutations have recently been identified [94, 95]. All missense mutations are located around or within the Aβ sequence. Mutations outside Aβ often lie clustered in close proximity to β- and γ-secretase cleavages sites and exert their pathogenic effect by influencing the activity of these secretases. These mutations alter Aβ production depending on their position, commonly resulting in increased Aβ production or increased ratios of $A\beta_{1\text{-}42/40}$ [96].

Intra-Aβ mutations *(Figure 4)*, in particular those close to the α-secretase cleavage site, seem to alter the Aβ sequence in a way that enhances its propensity to aggregate or result in protofibrillar Aβ formation [96]. Of these, only the Arctic *APP* (*APP*arc) mutation causes AD in a dominant manner. *APP*arc has been shown to enhance protofibril formation and has demonstrated plaque pathology without Congo red-positive Aβ cores in *post-mortem* examination [97, 98]. The A673V and the Osaka mutations cause AD in a recessive manner. Both show effects on Aβ production in accordance with their position; A673V enhances Aβ production, while the Osaka mutation enhances oligomerisation without fibrilisation [94, 95].

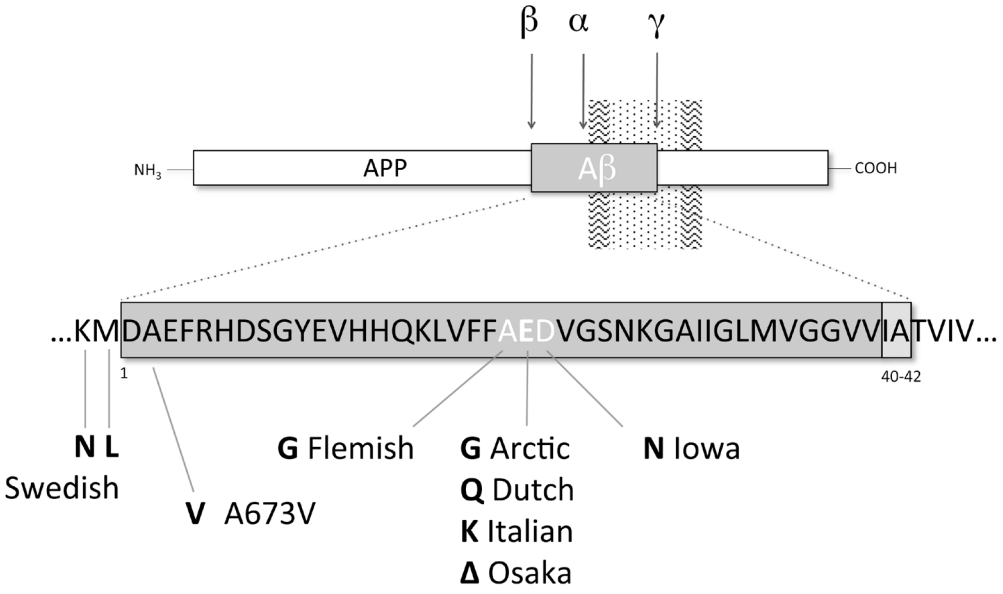


Figure 4. Location of intra-Aβ *APP* mutations and the Swedish *APP* double mutation.

PSEN1 and 2

Four years after the discovery of APP, genes on chromosome 14 and chromosome 1 were linked to familial AD and subsequently called presenilin 1 *(PSEN1)* and presenilin 2 *(PSEN2)* [89, 91]. Ever since, over 170 pathogenic *PSEN1* and 14 *PSEN2* mutations have been identified. Both genes have a very similar genetic structure, encoding two highly homologous proteins. PSENs constitute one of four components of *y*-secretase, a major player in Aβ generation. *PSEN* mutations cause alterations in the *y*-secretase cleavage site, commonly resulting in increased A$\beta_{1\text{-}42/40}$ ratios [96]. Interestingly, a dichotomous phenotype depending on the position of a certain mutation has been described. Mutations before codon 200 seem to be associated with younger age of onset and somewhat different pathology than mutations occurring beyond that point [99].

Sporadic AD

Several susceptibility genes have also been identified in connection with sAD. The most consistently associated is *ApoE*, encoding apolipoprotein E, which is involved in cholesterol metabolism. *ApoE* exists in three isoforms ε2, ε3, and ε4. The ε4 allele, by unknown mechanisms, appears to shift age of onset towards an earlier age and increases the risk of

developing AD. Heterozygous carriers of this allele have been shown to have a threefold risk, and homozygous carriers even up to a 15-fold risk of developing AD in comparison with ε3 homozygotes [100].

The four genes described so far have been estimated to account for less than 30% of the genetic variance in eoFAD and sAD/loAD [101]. The search for further genetic factors has identified several candidates, for example, *SORL1*, which has been found to cause loAD [102].

Clinical diagnosis

Dementia is commonly classified by using the criteria of the *Diagnostic and Statistical Manual of Mental Disorders,* fourth edition (DSM-IV) [103]. AD can at present only be diagnosed definitively *post-mortem* by histological confirmation. A clinical diagnosis can thus only be probable AD. For that, a history of the time course and character of symptoms is taken from the patient or the patient's social environment to assess whether cognitive or other instrumental functions are impaired and neuropsychological assessment is performed. The diagnosis is then made on the basis of criteria outlined by the National Institute of Neurologic and Communicative Disorders and Stroke – Alzheimer's Disease and Related Disorders Association (NINCDS–ADRDA) [104] according to which the diagnosis is classified as possible (atypical clinical features but no alternative diagnosis apparent without histological confirmation), probable (typical clinical syndrome without histological confirmation), or definite (clinical diagnosis with histological confirmation). Clinical AD diagnosis based on these criteria has proven sensitivity (distinguishing between AD patients and non-demented subjects) and specificity (distinguishing between AD and other forms of dementia) varying from 65% to over 80% and 23–88%, respectively [105, 106].

Several tools are used to stage AD in a clinical setting, such as the Mini Mental State Examination (MMSE) [107], the Clinical Dementia Rating (CDR) scale [108], and the Alzheimer's Disease Assessment Scale - cognitive subscale (ADAS-cog). The MMSE consists of a set of 30 items dealing with orientation, calculation, memory function, language abilities, attention and visuospatial function and provides a rough measure for a classification into no dementia, mild dementia, and moderate dementia [109, 110].

The CDR scale rates impairment in six functional categories (memory, orientation, judgement and problem-solving, community affairs, home and hobbies, and personal care)

on a five-point scale. The rating stretches from CDR 0 (no dementia), over CDR 0.5 (questionable dementia), CDR 1 (mild dementia), and CDR 2 (moderate dementia) to CDR 3 (severe dementia) [111].

The ADAS-cog is a more complex scale tapping eleven items that assess impairment of memory, language and praxis on a range of scores from 0 to 70 scores [87]. It is considered to be a thorough brief exam for the study of memory and language skills and therefore frequently used as a primary outcome measure in treatment trials in patients with mild to moderate AD [112, 113]. The results of a recent study suggested the use of sub-settings with different combinations of ADAS-cog test items to improve sensitivity to treatment effects [113].

Nowadays, the clinical diagnosis of AD is increasingly assisted by the use of several biomarkers; this is discussed in detail below (see "Biomarkers" and “Revision of diagnostic criteria”).

Neuropsychological assessment

In addition to the previously described rather unspecific assessments, numerous neuropsychological tests are applied to pinpoint impairment in different cognitive domains. Differences between normal aging and AD are especially obvious in episodic memory, semantic knowledge, and executive functions, but the syndrome of dementia also involves deficits in language, abstract reasoning, attention, and visuospatial abilities [114, 115]. Of the above, episodic memory is impaired earliest in AD [116]. This domain is heavily dependent on the functionality of brain structures in the medial temporal lobe, which are commonly affected by AD pathology in very early disease stages [26, 117].

No consensus has been reached on internationally standardised test batteries for neuropsychological testing in AD. However, in an attempt to systematise the assessment of patients and cognitively healthy individuals across the American Alzheimer's Disease Centers (ADC), the National Institute on Aging (NIA) recently designed a proposal for a uniform neuropsychological test battery [118]. This battery evaluates dementia severity by MMSE [107], attention by Digit Span (Wechsler Adult Intelligence Scale-Revised, WAIS-R [119]), processing speed by Digit Symbol (WAIS-R) and Trail Making Test A [120], executive function by Trail Making Test B [120], memory by Logical Memory, Story A, Immediate and Delayed Recall (WAIS-R), and language abilities by animal and vegetable list generation, and the Boston Naming Test [121].

Mild cognitive impairment

Mild cognitive impairment (MCI) is a more advanced form of age-related cognitive decline in which people notice subjective memory problems, and neuropsychological tests often confirm problems with delayed recall, although non-memory-related cognitive domains may also be impaired [122, 123]. The term MCI was introduced in 1988 but its definition has ever since been revised to characterise the subtle differences between MCI and early dementia, focussing on the fact that MCI describes a heterogeneous group of patients and that not all forms of MCI may convert to AD [123-125].

Individuals experiencing this transitional state between normal ageing and dementia are still able to live independently, but have an increased risk of developing dementia. MCI is classified as CDR 0 or 0.5 and furthermore subdivided into amnestic and non-amnestic MCI, depending on whether memory impairment is or is not involved in the clinical picture. Overall conversion rates to AD have been reported to range from 27% to 49%, depending on the subtype of MCI [126]. Annual progression rates have varied between 6–10% in epidemiological studies and 10–15% in clinical referral settings [126-128].

Biomarkers of AD

Biomarkers (or biological markers) are defined as indicators of biological processes, normal or pathogenic, that can be measured objectively to evaluate disease state or progression, guide clinical diagnosis, or monitor therapeutic interventions [129]. Potential treatment effects are very likely to be most if at all efficient at very early, preferably preclinical disease stages as much of the typical AD pathology forms years before clinical symptoms arise. The major aim of biomarker research is thus besides assisting a reliable clinical diagnosis of AD, to characterise early disease processes and put these in context to each other.

Neuroimaging

Neuroimaging with advanced techniques such as positron emission tomography (PET), structural and functional magnetic resonance imaging (MRI), and single photon emitting computed tomography (SPECT), has gained immense importance as a tool for diagnosis, treatment evaluation, and research into AD during the recent decade. Several

breakthroughs and paradigm shifts in AD research have been based on findings of neuroimaging studies.

Magnetic resonance imaging

Magnetic resonance (MR) brain imaging is widely used in assisting the clinical diagnosis of AD and is a well-established tool in AD research. Good availability, the non-invasive nature, and relatively low costs are important advantages of this technique. The actual MRI technique embraces a variety of different applications, which can roughly be divided into structural (volumetric, diffusion-weighted, diffusion tensor (DTI)), functional (perfusion, blood oxygenation level-dependent (BOLD) functional MRI (fMRI), arterial spin labelling (ASL)), and spectroscopic (MRS) applications. Structural MRI, especially measures of volumes and cortical thickness, has so far been the most-used application of this technique in AD, based on the fact that atrophy as assessed by MRI and neuronal loss as well as NFT pathology are highly correlated [130, 131].

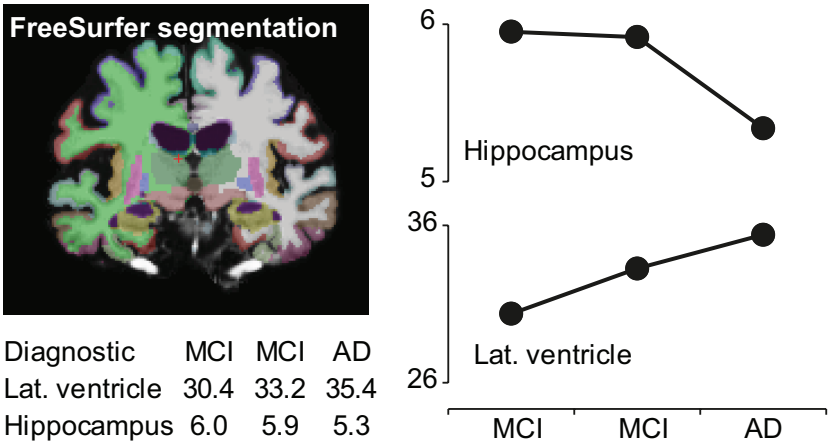


Figure 5. Automated segmentation results for longitudinal structural MRI scans in an MCI patient progressing to AD (Courtesy Dr Spulberg, Karolinska Institutet).

Atrophy of the medial temporal lobe, more specifically the hippocampus and the entorhinal cortex, has consistently been shown to distinguish between AD patients and healthy controls with high sensitivity and it progresses more rapidly in AD patients and in individuals at risk of sAD [132-136]. Manual outlining of these regions is time-consuming, and this is why several automated procedures have been developed and refined. *Figure 5* shows an example for results from an automated segmentation as implemented in the FreeSurfer software package (http://surfer.nmr.mgh.harvard.edu).

Structural MRI studies in eoFAD mutation carriers are rare, some have been suggesting that atrophic changes occur closer to the expected age of AD onset than, for example, a decrease in glucose metabolism [137, 138]. Of interest is a recent study that revealed increased entorhinal cortical thickness in *PSEN1* mutation carriers about 10 years prior to expected disease onset, suggesting inflammatory processes underlying the hypertrophy [139].

An interesting novel approach is the combination of structural or fMRI with amyloid imaging by means of PIB PET (see "PIB in AD: Amyloid") to assess the disruption of neuronal networks in relation to regional fibrillar amyloid burden [140, 141].

Positron emission tomography

In the early 1950s, the first experimental positron imaging devices were developed to improve imaging of brain tumours and other brain disorders [142]. Gradual improvement of apparatus and technique finally led to today's positron emission tomography (PET), an unparalleled method providing measurements of functional and pathological processes *in vivo*. A PET examination requires highly specialised equipment and personnel and is therefore often put in competition with less costly and better accessible functional imaging methods, such as fMRI or SPECT. Yet PET can not only image (visualise) a broad variety of functional processes but also quantitatively measure them, a unique feature that is not shared by other methods.

Principles of PET

PET is a tomographic modality (*tomography: "A method of producing a three-dimensional image of the internal structures of a solid object (such as the human body [...}) by the observation and recording of the differences in the effects on the passage of waves of energy impinging on those structures."* [143]) for imaging the spatial and temporal distribution of radioactive tracers (ligands, biological indicators) at a molecular level. These tracers are labelled with positron emitting radionuclides (isotopes) with short half-life *(Table 2)*, which are produced in a particle accelerator called a cyclotron.

Isotope	Half-life (min)	Characteristics
^{15}O	2.05	Used in blood flow/activation studies
^{11}C	20.4	Replaces natural carbon in molecules (many application areas)
^{68}Ga	68.3	Used for transmission scan (tissue attenuation correction)
^{18}F	110	Long half-life (better availability for sites without cyclotron)

Table 2. Isotopes typically used in brain PET.

To create a tracer, a radionuclide is chemically attached to a specific tracer molecule, which is administered systemically and distributed by the blood to its specific target. There, the tracer undergoes β-decay, emitting positrons that are annihilated by electrons, in turn producing two 511 keV photons (γ rays) at 180° to each other. Scintillation detectors (crystals) detect the coincidental photon impacts and the location of the decay can be reconstructed along the line of response (LOR) *(Figure 6)*.

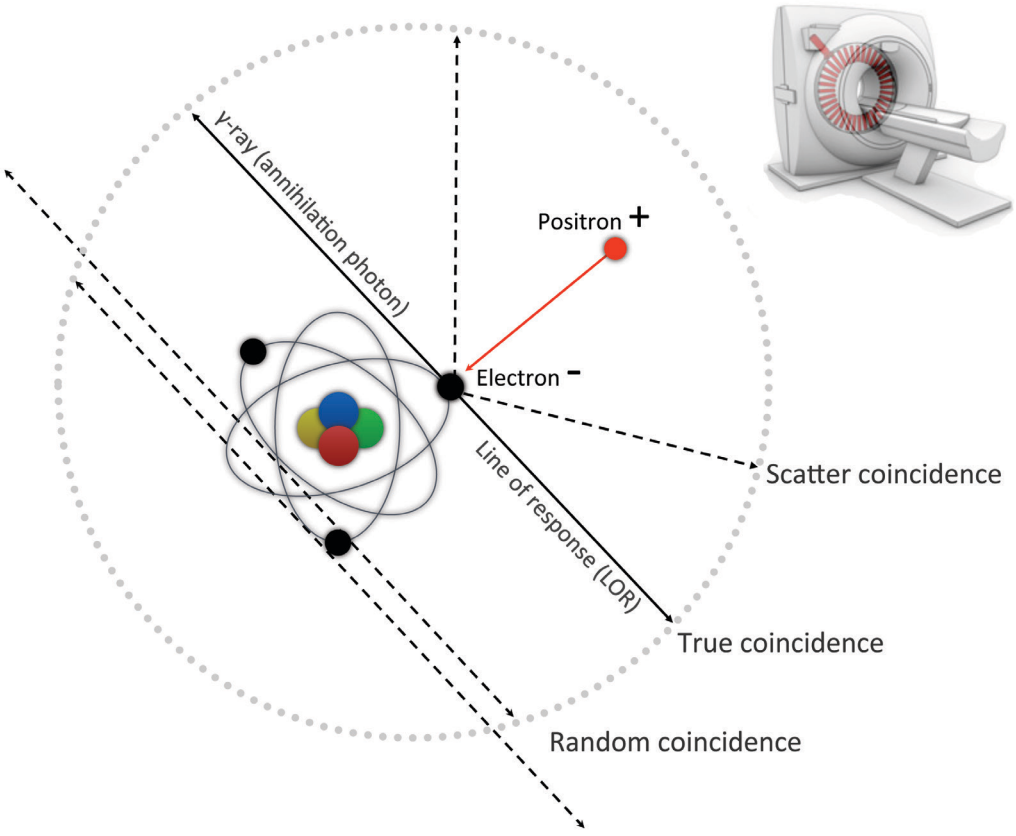


Figure 6. Basic principles of PET.

Multi-tracer PET approach

PET is a molecular imaging technique, providing *in vivo* insights in physiological and pathological processes at a molecular level. Multi-tracer PET, the combination of different tracers allows the observation of, for example, disease-specific changes in several systems by gathering complementary information on these processes. As for AD, many aspects of its pathology are known or suggested to start early and show dynamic progress. PET using multiple tracers can detect many of these changes *in vivo* and describe them from different angles. PET tracers are radiolabelled molecules that can have hugely varying binding capacities and sites and thus explore an equal amount of potential targets. The choice of a respective radionuclide *(Table 2)* is based on specific needs, shorter half-lives enable multiple consecutive studies, whereas longer half-lives can be of use if, for example, slower metabolic processes are to be studied. Of great benefit is the use of multi-tracer PET in the evaluation of potential treatment efficacy. It enables monitoring of different effects a treatment has on molecular mechanisms in reduced sample sizes, as examples from our group have shown where, for example, the effects of AChEI treatment on nicotinic binding site density, AChE activity, glucose metabolism, and blood flow were measured [144-147].

In this work, a multi-tracer PET approach was employed combining tracers for the study of glucose metabolism, monoaminoxidase-B activity as a marker for astrocyte activation, and a tracer for fibrillar amyloid pathology *(Figure 7)*. These tracers and the study aims are described later.

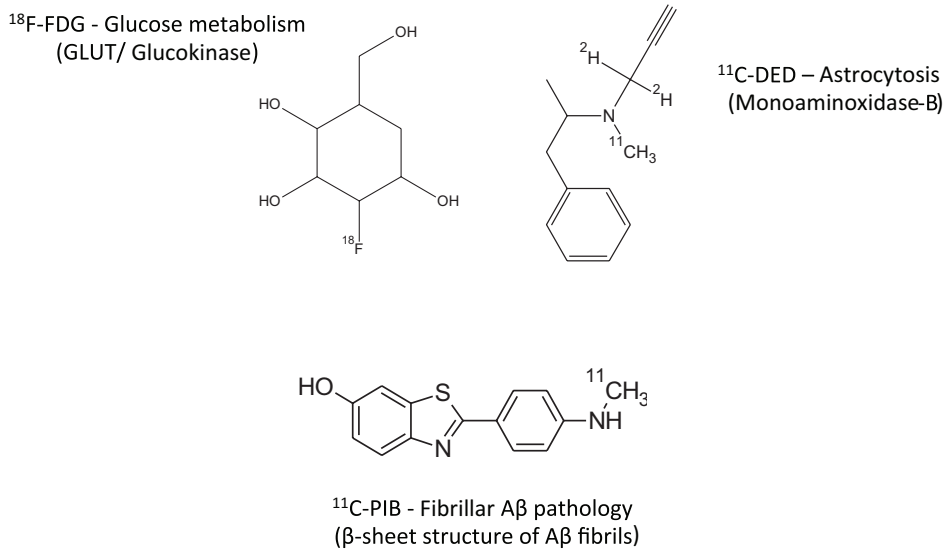


Figure 7. Multi-tracer approach employed in this work.

Some general aspects should be considered here:

- Comparison between different studies can be complicated by variations in standards of processing, modelling, and analysis of PET data.
- Effects of several kinetic aspects on tracer delivery, distribution, and binding (e.g. blood flow component, atrophy in AD) must be taken in account.
- For some tracers, the target is well characterised but not the actual binding site and its features.

PET scanner

Early positron imaging devices had scintillation detectors gathered in two arrays opposite to each other. Modern PET scanners feature hexagonal, octagonal or fully circular detector arrays with commonly 12-15 cm field of view (FOV) and resolutions of 4-5 mm full-width half-maximum (FWHM) [148]. A dedicated high-resolution research tomograph (HRRT) can achieve up to <2 mm FWHM in resolution [148, 149]. Nowadays, many PET scanners are combined with structural imaging devices such as X-ray computed tomography (CT), and recently also PET/MRI combinations have become commercially available. Last generation PET scanners also feature very high timing resolution that enables time-of-flight (TOF) reconstruction. TOF allows correction for tiny timely differences in the detection of each annihilation photon along the LOR [150].

Data acquisition and processing

PET examinations are either performed as static scans or as dynamic time series with several time frames. Dynamic PET imaging involves a sequence of contiguous acquisitions upon tracer injection, each of which can last between 10 s and 20 min, depending on the used tracer. Upon acquisition, the radiation data from the camera is corrected for tissue attenuation and scatter, reconstructed, and finally corrected for motion.

Data visualisation and modelling

The quantitative image resulting from data processing displays radiation values for each voxel (Bq/voxel) in a scanned volume. The most basic analysis is a visual evaluation either of the individual time frames or of a summation image of several (usually late)

frames. In a next step, ratios can be calculated by measuring tracer activity in a defined region or volume of interest (ROI/VOI) and dividing other ROIs/VOIs or global activity by the values obtained from the reference region. The Standardized Uptake Value (SUV) is calculated by dividing each voxel's radioactivity at a certain time point by the injected dose (Bq) and the person's body weight (kg) [151]. Ratios and SUVs are commonly used for simple measures of biodistribution or characterisation of diseases.

More complex mathematical models have to be applied for the calculation of certain physiological parameters. To relate local tracer activity to biochemical processes, tracer transfer between different compartments has to be modelled. These compartments represent either anatomical spaces that can not be visualised by scanner resolution or different biochemical states of the tracer in the same site [152]. These models require an input function, a measure of the arterial tracer concentration that is available to the brain. Arterial blood samples are ideally used for calculating the input function but cause significant inconvenience for the proband. This is why in many cases, especially for 2-[^{18}F]-fluoro-2-deoxy-D-glucose (FDG) PET scans, "arterialised" venous blood from a heated hand vein is used [153]. Other possibilities for creating an input function are the definition and application of a reference region or the direct measurement of arterial tracer activity on high-resolution PET images [152].

Quantitative regional analysis

Accurate anatomical PET data analysis is aggravated by the rather poor spatial resolution of standard PET scanners (4-5 mm FWHM), which exceeds e.g. average brain cortical thickness [154]. Manual outlining of cortical ROIs/VOIs *(Figure 8A)* directly on the processed PET images is thus a fairly crude method that may be guided by signal "hotspots" rather than by actual anatomy and leads to over-/underestimation of detailed regional data. Better anatomical accuracy is achieved by placement of ROIs/VOIs on the individual's coregistered MRI scan. A confounding factor is hereby the "spill-over" of tracer signal from other tissue compartments, such as white matter or CSF, into the grey matter, called partial volume effect [155]. Especially in disorders like AD, where the brain is subject to massive atrophy, outlining ROIs/VOIs without partial volume correction often leads to an underestimation of regional signal due to partial volume effects [156].

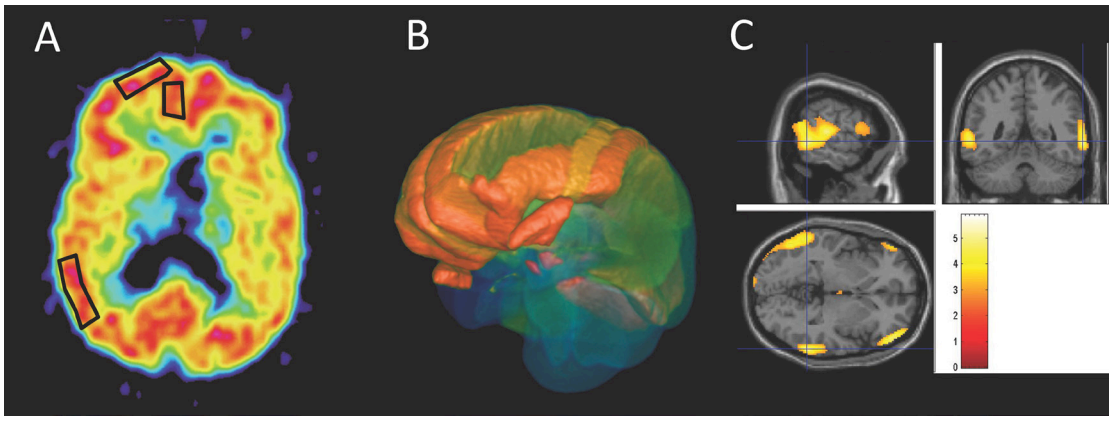


Figure 8. Different methods for PET data analysis. (A) Manual outlining of ROIs directly on slices of PET images. (B) Predefined atlas (exemplified by a three-dimensional rendering of the "Hammersmith" atlas based on 30 segmented MRI scans [157]), which is applied onto a PET scan in the same space. (C) Colour coded SPM T map showing results from a group comparison projected on an MRI template.

The probably most accurate method for regional analysis constitutes the application of a predefined anatomical atlas *(Figure 8B)* after spatial normalisation of either the PET scan or the atlas guided by the corresponding MRI scan.

Voxel-based analysis based on the general linear model as provided by the free software package SPM (Statistical Parametric Mapping, Wellcome Institute, London, UK), is particularly useful when PET data from groups are compared to each other. In short, individual PET scans are spatially normalised to a template, followed by voxel-by-voxel (mass-univariate) comparison between the means of the two groups. The resultant statistics are then presented in a statistical parametric map *(Figure 8C)*.

PET in AD

The first PET studies in AD patients date back to the early 1980s. One of the earliest used ^{15}O to study cerebral oxygen supply [158], following studies used ^{18}F-FDG PET to assess glucose metabolism [159-161]. Since then, many more compounds have come along and countless PET scans have been performed in the study of AD. *Table 4* provides an overview of PET tracers that have been or are in use in AD research.

Blood flow

Most nuclear imaging studies assessing cerebral perfusion or blood flow in AD have made use of SPECT, showing decreased perfusion in brain regions that typically also display

impaired glucose metabolism (see "Glucose metabolism"), such as posterior cingulate and parietotemporal cortical regions [162, 163]. Some studies have used PET with ^{15}O-H_2O to evaluate perfusion, a tracer with a half-life of merely 2 min, which enables acquisition of multiple data sets over a brief period but requires on-site production and fast handling [164, 165]. Separate PET examinations to study cerebral blood flow might though be rendered unnecessary by making use of the early time frames of e.g. FDG and/or PIB PET scans, reflecting the tracer delivery phase by blood, as a measure of perfusion [166-168].

Glucose metabolism

Glucose utilisation in tissue can be measured by 2-[^{18}F]-fluoro-2-deoxy-D-glucose (FDG) PET. FDG, a radiolabelled glucose analogue, is actively transported into cells by a group of transport proteins (GLUT) and phosphorylated by hexokinase to FDG-6-PO_4, which in contrast to phosphorylated natural glucose can not be further metabolised via glycolysis [169]. It is therefore essentially trapped in cells at least for the duration of an average PET scan of 45 to 90 min.

FDG PET has been used to evaluate deterioration in the AD brain for almost 30 years [159-161] and is certainly the best-evaluated PET tracer in AD research, increasingly also used in the clinical diagnosis of AD. Against a background of global hypometabolism, numerous studies have reported a specific decrease in glucose metabolism in the parietotemporal association and angular and posterior cingulate cortices, as well as the medial temporal lobe including structures such as the hippocampus and entorhinal cortex, whereas regions like the cerebellum, pons, striatum, sensorymotor and primary visual cortices remain spared in sAD patients [170-172]. This pattern can vary with age of disease onset and disease severity. One study revealed more severe hypometabolism in parietal, frontal, and several subcortical areas, while in another study greater impairment in parietal and posterior cingulate cortices and precuneus in eoAD when compared with loAD was reported [173, 174]. The diagnostic accuracy of FDG PET as regards a definite AD diagnosis has been shown to be over 90% in terms of sensitivity in pathologically confirmed AD, exceeding previous clinical diagnostic accuracy significantly [175, 176].

The decrease in cerebral glucose metabolism (CMRglc) is closely related to cognitive decline [177-179] and hence has consistently been shown to be a reliable predictor for the progression from healthy ageing to MCI and from MCI to AD.

Hypometabolism mainly in the posterior cingulate cortex and medial temporal lobe, more precisely the hippocampus, but also in the parietotemporal cortex has figured as a robust measure of disease progression [180-185]. Furthermore, CMRglc reductions in the angular, left mid-temporal, and left middle frontal gyri have been shown to be associated with faster cognitive decline among cognitively healthy individuals [186]. Likewise, hypometabolism, albeit mild, has been observed in the same regions as in clinically affected AD patients in non-demented individuals carrying at least one *ApoE* ε4 allele compared with non-carriers and has been shown to be progressive and to correlate with cognitive performance in these individuals [187-190].

A few studies have assessed the pattern of hypometabolism in pre-symptomatic carriers of eoFAD mutations, all of which demonstrated regional abnormalities prior to the development of cognitive impairment. The results of these studies varied somewhat, very likely due to the fact that they each embraced different eoFAD mutations at different time spans from their expected age of disease onset. In summary, a decrease was, often accompanied by global CMRglc impairment, most commonly observed in parietal, parietotemporal, temporal, posterior cingulate, and entorhinal cortical regions, but also in subcortical structures such as the hippocampus and the thalamus [137, 138, 191-194].

An interesting connection to mitochondrial dysfunction was established by the finding of declining glucose metabolism in brain regions typically affected in AD in individuals with a maternal, rather than paternal, family history of AD [195, 196]. This concept has further been validated by the observed correlation between *post-mortem* mitochondrial dysfunction and *in vivo* FDG PET measures in the posterior cingulate cortex [197, 198].

FDG PET has also been proved to be a valuable outcome measure in treatment studies [144, 147], permitting a substantial decrease in the required sample size in treatment trials [179, 199].

The FDG PET signal is commonly referred to as a surrogate marker of neuronal or synaptic integrity. It is still an unresolved issue to what extent astrocytic glycolysis accounts for that signal; this knowledge should contribute significantly to the understanding of measures of glucose metabolism by FDG PET [72, 73].

Neurotransmitters

In a complex disorder such as AD, all neurotransmitter systems are affected. In particular the acetylcholine system is impaired as a consequence of neuronal loss in cholinergic brain nuclei such as the nucleus basalis Meynert and decreased levels of acetylcholine (ACh) and acetylcholinesterase and acetyltransferase activity [200]. PET studies using tracers for AChE activity such as ^{11}C-MP4A and ^{11}C-PMP have shown decreased binding in AD and MCI patients when compared to normal controls [201]. Different tracers are under development for different subtypes of ACh (nicotinic and muscarinic) receptors, *Table 4* provides an overview of these compounds.

The PET tracer portfolio offers the possibility to study dopamin synthesis, storage, receptor binding, reuptake, or vesicular transporter of dopamine. In AD, striatal D1 receptors have been shown to be reduced in comparison with controls; D2 receptor binding potential, however, was decreased in the hippocampus and in temporal cortical regions [202].

Cognitive function has recently been associated with the serotonin transporter (SERT) [203], and a significant reduction in 5-HT2A receptor binding has furthermore been revealed in early MCI patients. These levels remained stable on a lower level than those in controls over a two-year follow-up, even in patients who converted to AD [204].

β-Amyloid

With Aβ being a central component of AD pathogenesis, the rise of PET imaging ligands that could visualise Aβ pathology was a minor revolution. The first amyloid tracer to come to use in 2002 [205], *N*-methyl-[^{11}C]-2-(4'-methylaminophenyl)-6-hydroxy-benzothiazole, called Pittsburgh Compound B (PIB), a thioflavin T derivate, is still the most widely used. PIB crosses the blood-brain barrier and binds with high nanomolar affinity to insoluble fibrillar aggregates of Aβ [206], predominantly to fibrillar neuritic plaques but also to vascular Aβ pathology in form of cerebrovascular amyloidogenic angiopathy (CAA) [207, 208]. It has also been shown to correlate well with measures of insoluble Aβ and ^{3}H-PIB binding in *post-mortem* brain tissue, amongst others from the first-ever patient to be scanned with PIB *in vivo* [207, 209, 210].

The first PIB PET study in humans, carried out in Uppsala in patients from the Karolinska University Hospital Huddinge and in collaboration with the Pittsburgh

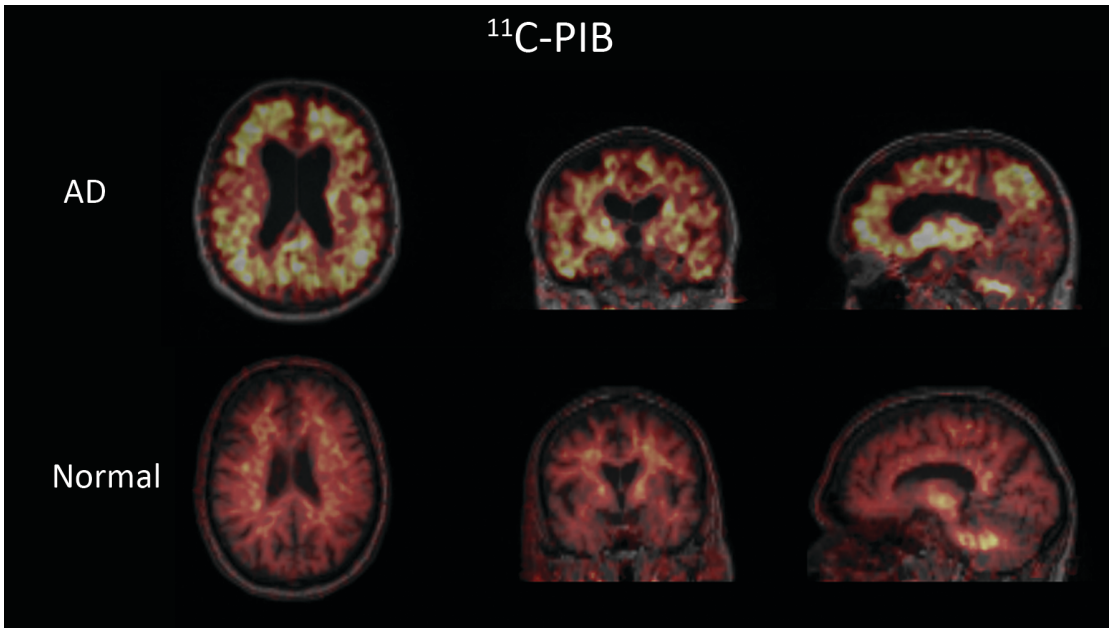


Figure 9. Representative ^{11}C-PIB PET scans of age-matched individuals.

group, found that PIB retention was significantly higher in the frontal and temporoparietal association cortices as well as in the striatum in AD patients compared with healthy controls [211]. PIB retention was similar in patients and controls in brain regions known to be relatively unaffected by amyloid deposition, such as the pons, the cerebellum and subcortical white matter. This differentiation could be replicated by several subsequent studies in AD patients and healthy individuals [212-214]. PIB retention seems to remain stable over time once AD is diagnosed [215, 216], a pattern well reflected by the poor correlation between FDG PET measures of decreasing glucose metabolism and PIB retention, although few regional correlations have been reported [216-219].

The pattern of PIB retention in MCI can be described as quite dichotomous. Those MCI patients who eventually will convert to AD show usually higher and temporally increasing PIB retention than those who will most probably remain stable or return to a more normal cognitive state [220]. Interestingly, increased PIB retention has also been demonstrated in a substantial amount of healthy elderly [214]. It has been argued that these individuals might be at an increased risk of developing cognitive decline.

With low CSF levels of Aβ being supposed to represent high Aβ brain deposition, a clear inverse relationship to PIB retention has been shown [219, 221]. However, several

cases have been described with abnormally low CSF Aβ levels and no elevated PIB detected by PIB PET [222, 223].

Genetic risk factors and causes of AD have furthermore been reported to predispose for PIB retention. Both cognitively normal elderly and AD patients carrying *ApoE* ε4 alleles revealed gene dosage dependent higher PIB retention levels than non-carriers [224, 225]. Studies in eoFAD mutation carriers demonstrated particularly high PIB uptake in the striatum, already at young pre-symptomatic and at symptomatic disease stages where also high cortical levels were observed [226-228].

The short half-life of ^{11}C renders the use of PIB distant from a cyclotron impossible. This is why several ^{18}F-labelled compounds have been developed *(Table 4)*. The first, ^{18}F-FDDNP was shown to bind to both fibrillar Aβ and tau pathology and to differ somewhat from PIB with regard to the dynamic range of retention [229, 230]. Recently, fluorinated tracers such as ^{18}F-Florbetaben [231] ^{18}F-Flutemetamol (also called F-PIB, similar structure) [232] ^{18}F-Florbetapir (also called ^{18}F-AV-45) [233] have demonstrated promising binding characteristic and diagnostic properties with regard to AD. The US Food and Drug Administration (FDA) has recently evaluated ^{18}F-AV-45 but not yet approved. Issues of clinical application in terms of AD assessment will have to be resolved before amyloid ligands can become part of a diagnostic routine.

Neuroinflammation

Microglia

PET ligands for *in vivo* visualisation of microglial activation make use of their high affinity to the peripheral benzodiazepine-binding site (PBBS/PBR), also called translocator protein (TSPO), a receptor located in the outer membrane of mitochondria. Its upregulation within the CNS has been shown to reflect neuroinflammatory processes, mainly due to the activation of microglia [234, 235]. The most used PET ligand to study microglial activation is the ^{11}C-labelled isoquinoline (*R*)-PK11195 (1-[2-chlorophenyl]-*N*-methyl-*N*-[1-methyl-propyl]-3-isoquinoline carboxamide) (PK11195), a specific ligand for TSPO. An early PK11195 PET study that did not show any difference between AD patients and a group of controls suffered from several methodological issues [236]. Subsequent PET studies used the aforementioned more active *R*-enantiomer of PK11195 and applied different and more advanced quantification approaches to

improve tracer evaluation [237, 238]. One of these found increased binding levels in the entorhinal, temporoparietal, and cingulate cortex in a group of AD patients and one subject with mild cognitive impairment (MCI) in comparison with normal individuals [239]. Several studies have combined PK11195 PET imaging with PET examination of fibrillar amyloid with PIB and dementia assessments to explore the relationship of microglial activation with underlying pathology in AD and MCI. One reported increased PK11195 binding in AD patients in comparison with normal controls in parietotemporal regions and a negative correlation with PIB retention levels in the posterior cingulate, which also showed lowest glucose metabolism as measured by ^{18}F-FDG PET [240]. Another study found increased PK11195 cortical binding and a two-fold elevated PIB retention in the same cortical areas of AD subjects when compared to healthy controls [241]. A study in 14 MCI patients showed that half of them had increased cortical PIB retention while only five had increased PK11195 levels, and no regional correlation between the tracers was found [242]. No difference in PK11195 binding between mild to moderate AD patients, MCI patients and control subjects and no regional correlation with PIB retention were found in once another study claiming that microglial activation might be associated with later stages of AD alone or that PK11195 might be too insensitive to detect microglial activation at the examined disease stages [243]. This is, however, in disagreement with a study showing increases in microglial activation even during healthy aging [244].

Even if PK11195 is still considered the "gold standard", the results of PET studies in AD and MCI have been rather discordant, especially in earlier disease stages. New tracers such as *N*-(2,5 dimethoxybenzyl)-*N*-(4-fluoro-2-phenoxyphenyl) acetamide (DAA1106) have higher binding affinity to TSPO and binding characteristics superior to PK11195. One study has so far been conducted in AD showing significantly higher binding in cortical regions, as well as in the striatum and the cerebellum of AD patients compared with controls [245]. Another TSPO ligand, ^{11}C-vinpocetine, has also been suggested as a potential marker for microgliosis. No difference between AD patients and age matched control subjects was observed, however, disease and age specific changes could successfully been demonstrated [246]. Interestingly, two different TSPO binding sites with different affinities have recently been identified, which might explain the variation in the PET studies using TSPO tracers [247].

Category	PET tracer	Measures
Functional	^{18}F-FDG	Glucose metabolism
	^{15}O-H_2O	Blood flow
Acetylcholine	^{11}C-PMP	AChE activity
transmitter	^{11}C-MP4A	AChE activity
system	^{11}C-Nicotine	Nicotinic ACh receptor levels
	^{18}F-Fluoro-A-85380	Nicotinic ACh receptor levels
	^{11}C-NMPB	Muscarinic ACh receptor levels
	^{11}C-Benztropine	Muscarinic ACh receptor levels
	^{11}C -CHIBA-1001	α7 nicotinic receptor levels
Dopamine	^{18}F-Fluordopa	Dopamine synthesis and vesicular storage
transmitter	^{11}C-SCH-23390	D_1 receptor levels
system	^{11}C-Raclopride	D_2 receptor levels
Serotonine	^{11}C-MPPF	5-HT1A receptor levels
transmitter	^{18}F-Altanserin	5-HT2A receptor levels
system	^{11}C-SB207145	5-HT4
	^{11}C-DASB	SERT
Pathology	^{11}C-PIB	Fibrillar amyloid
	^{18}F-Flumetamol	Fibrillar amyloid
	^{18}F-Florbetapir (AV-45)	Fibrillar amyloid
	^{18}F-FDDNP	Fibrillar amyloid / tau
	^{11}C-SB-13	Fibrillar amyloid
	^{11}C-BF-227	Fibrillar amyloid
	^{18}F-THK523	Tau (experimental)
Inflammation	^{11}C-(R)-PK11195	Translocator protein system (TSPO), Microglia
	^{11}C-DAA1106	TSPO, Microglia
	^{11}C-Vinpocetine	TSPO, Microglia
	^{11}C-L-Deprenyl (DED)	Monoaminoxidase B (MAO B), Astrocytes

Table 4. PET tracers (trivial names) used in AD research.

Astrocytes

^{11}C-L-deuterium-deprenyl (DED) binds to monoamine oxidase B (MAO-B), an enzyme abundant predominantly in the mitochondria of astrocytes and in serotonergic neurons in the human brain [248-250]. Enhanced MAO-B activity has been related to astrocytic activity, in *post-mortem* tissue mostly associated with Aβ plaque pathology [251-253]. DED PET has been used in the study of amyotrophic lateral sclerosis [47] and

Creutzfeldt-Jakob [48] disease, amongst others, demonstrating increased DED binding in the brains of patients in comparison with controls. Very recently, the first study involving AD patients and healthy controls has been published. This study revealed no difference in DED binding between a group of AD patients and healthy controls, however, after applying a binding over tracer distribution ratio that was considered to correct for differences in blood flow between patients and controls, the AD patients showed higher values of this ratio in frontal, parietal, and temporal lobes [254]. Pure DED binding did not correlate with PIB retention, although ratio values correlated with PIB retention in the occipital cortex (see also "Results & Conclusion").

Tau

So far, no study has been published concerning the use of a PET tracer specific for tau pathology in humans. There are though several candidate ligands [255], of which at least one, ^{18}F-THK523, has been studied in transgenic mice, with promising results, and it is currently being studied in AD patients [256].

Cerebrospinal fluid biomarkers

Cerebrospinal fluid (CSF) is a bodily fluid present in the ventricular system within and around the brain and in the central canal of the spinal cord. It is accessed and acquired by lumbar puncture, a relatively safe and cost-effective procedure that can be carried out without the need of advanced technical apparatus [257]. CSF biomarkers are believed to reflect molecular and metabolic changes in the extracellular and interstitial environment of the brain given the almost unrestricted flow of molecules towards the CSF [258].

CSF biomarkers for AD should ideally depict typical AD pathology. Among the large collection of candidates, the best established and most widely used are thus measures of $A\beta_{1-42}$, total tau (t-tau) and phosphorylated (at threonine 181) tau (p-tau). Levels of $A\beta_{1-42}$ are commonly decreased in AD, likely due to its deposition in plaques in the brain [259, 260], while levels of t- and p-tau are significantly elevated. T-tau thereby possibly reflects the amount of neuronal degeneration [261-263], while p-tau rather correlates with neurofibrillary tangle burden [258, 264].

The combination of the three biomarkers, rather than one alone, has been proven to distinguish AD from cognitively healthy individuals with high sensitivity (generally

~90%) in numerous studies [223, 265-267]. Most longitudinal studies in AD patients have reported rather stable levels of $A\beta_{1-42}$ and tau over time, although some showed fairly conflicting results [268-272]. Highly consistent, however, were results from the many studies evaluating how well the CSF biomarker troika predicts conversion from MCI to AD, reaching sensitivity values of up to 95% [265, 273-275]. Interestingly, only changes in CSF $A\beta_{1-42}$, or t-tau or p-tau/$A\beta_{1-42}$ ratio, not tau alone, predicted cognitive decline or eventual progression to AD in cognitively healthy individuals [276-279].

Studies in carriers of different *PSEN1* eoFAD mutations showed that pre-symptomatic mutation carriers had either decreased CSF $A\beta_{1-42}$ levels or $A\beta_{1-42/40}$ ratios or showed that $A\beta_{1-42}$ levels correlate with time to disease onset [280-282]. CSF levels of tau were either elevated at pre-symptomatic stages or at later symptomatic stages, correlating then with disease severity [280, 282]. One study furthermore studied $A\beta$ species patterns in mostly pre-symptomatic carriers of a *PSEN1* mutation and sAD patients and found that levels of $A\beta_{1-42}$ and $A\beta_{1-16}$ were similarly low in eoFAD and sAD, while the mutation carriers had very low levels of $A\beta_{1-37}$, $A\beta_{1-38}$, $A\beta_{1-39}$, suggesting modulation of the γ-secretase cleavage site by the *PSEN1* mutation [283]. Another study followed a very young pre-symptomatic *APP* mutation carrier longitudinally, reporting changes in both $A\beta_{1-42}$ and tau CSF levels whereof levels of p-tau changed most over the 4.5 y follow-up period [284].

With three profoundly established core biomarkers and many candidates in the pipeline, CSF measures have shown encouraging results in numerous studies. However, it is important to consider that significant intercenter and interlaboratory variations have occurred in large multicenter studies [285, 286]. This is why great efforts are now undertaken to standardise operation procedures and increase the accuracy of measurements at different sites [287, 288].

Revision of the diagnostic criteria for AD

Based on the above-mentioned findings, Dubois and colleagues drafted in 2007 (on behalf of an international working group) new research criteria for the diagnosis of AD that were no longer based on exclusionary conditions but characterised by phenotypes [289]. The core innovation was based on the extended use of biomarkers, which had been subject to extensive research during previous years. The revised diagnosis of probable AD thus demanded the presence of episodic memory impairment together with so-called supportive

features such as the presence of medial temporal lobe atrophy assessed by MRI, abnormal concentrations of typical markers in CSF, or pathological patterns on functional neuroimaging by means of PET. A series of exclusion criteria was also specified. The revised criteria for definite AD included either the combination of clinical and histopathological or clinical and genetic evidence.

Two recent reports have evaluated the accuracy of these revised diagnostic criteria. In a retrospective study in 243 subjects with *post-mortem*-confirmed diagnosis, 68% sensitivity and 93% specificity were achieved when memory and CSF data with exclusion criteria were applied, and a specificity of 100% was reached when supportive features were included [290]. In another study the records of 150 patients were re-evaluated [291]. Here, 55% of all patients previously diagnosed with AD, met the revised diagnostic criteria for probable AD. Among a group of non-demented subjects, 7.3% were diagnosed as having AD. Of note is that none of the subjects included in the two studies had undergone all recommended examinations.

In 2009, the NIA established three international working groups to formulate diagnostic criteria that would, similar to the approach Dubois *et al.* followed, incorporate all scientific advances made since the introduction of the original NINCDS-ADRDA criteria [292]. The three groups were to focus on the asymptomatic, preclinical phase, the symptomatic pre-dementia phase, and the dementia phase of AD, respectively. The new criteria for each of these phases were recently published in Alzheimer's & Dementia, the organ of the Alzheimer's Association [293-295]; they are summarised in *Table 5*.

How well these new criteria will work in clinical practice is yet to be explored. Although there is an undisputed use in including well-established biomarkers of AD in the diagnostic process, not all patients will have the infrastructural or financial possibilities to undergo all or even some of the suggested examinations.

Diagnostic category	Subtle cognitive changes	Biomarker probability of AD aetiology	Aβ (PET or CSF)	Neuronal injury (CSF tau, FDG-PET, structural MRI)
Preclinical stage 1	Negative		Positive	Negative
Preclinical stage 2	Negative		Positive	Positive
Preclinical stage 3	Positive		Positive	Positive
MCI–core clinical criteria		Uninformative	Conflicting/ indeterminate/ untested	Conflicting/ indeterminate/ untested
MCI due to AD—intermediate likelihood		Intermediate	Positive Untested	Untested Positive
MCI due to AD—high likelihood		Highest	Positive	Positive
MCI—unlikely due to AD		Lowest	Negative	Negative
Probable AD dementia Based on clinical criteria		Uninformative	Unavailable, conflicting, or indeterminate	Unavailable, conflicting, or indeterminate
With three levels of evidence of AD pathophysiological process		Intermediate	Unavailable or indeterminate	Positive
		Intermediate	Positive	Unavailable or indeterminate
		High	Positive	Positive
Possible AD dementia (atypical clinical presentation) Based on clinical criteria		Uninformative	Unavailable, conflicting, or indeterminate	Unavailable, conflicting, or indeterminate
With evidence of AD pathophysiological process		High but does not rule out second aetiology	Positive	Positive

Table 5. Revised diagnostic criteria for AD (based on [293-295]).

Time course and interrelationship of biomarkers

The complex interplay of pathological processes underlying AD has been explored in multitudinous studies employing *in vivo* biomarkers, some of which are described above. Many of these studies have been conducted in different settings, with diverging patient characteristics and greatly varying methodology, which poses complications in comparing their results. As a general conclusion, however, it can be stated that the combination of different biomarkers not only improves the understanding of disease mechanisms but will also be necessary to eventually arrive at reliable clinical diagnosis. It is, however, not necessarily decisive to combine all available biomarkers to achieve the highest classification rates but rather to know at which stage of disease development a certain combination of biomarkers might be most useful.

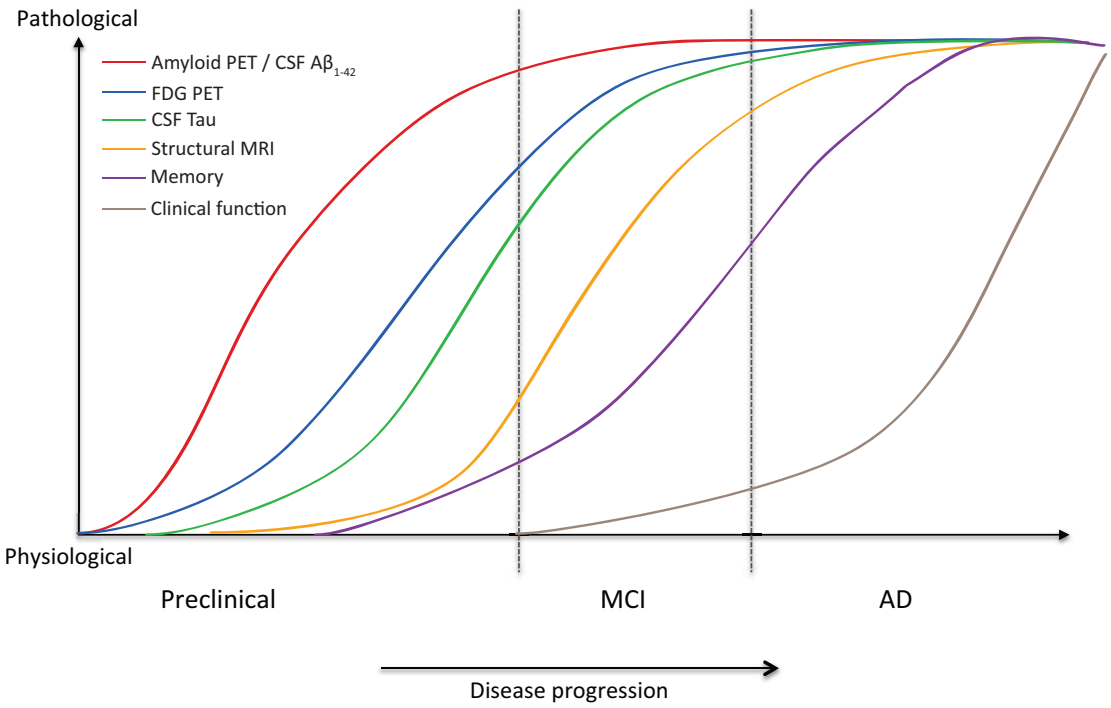


Figure 10. Hypothetical time course of AD biomarkers (based on [293, 296-299].

In an attempt to confirm findings from previous studies in standardised, large-scaled settings, and to set methodological standards initiatives such as the Alzheimer's Disease Neuroimaging Initiative (ADNI) [300], the Australian Imaging, Biomarkers and Lifestyle (AIBL) study [301] for sAD, as well as the Dominant Inherited Alzheimer Network (DIAN) for eoFAD, have been established. ADNI has been ongoing for the past six years and resulted in many publications on the interrelationships of biomarkers, whereas AIBL and DIAN have quite recently commenced. While benefits and impact are undisputed,

there are limitations to these large-scaled initiatives. Both ADNI and AIBL have gathered groups of AD and MCI patients, as well as healthy controls aged on average well over 70 [301, 302]. At this age, many pathological processes might already have been ongoing for years. The cumulated knowledge from currently available studies on AD biomarkers can be presented in the form of time course of the individual biomarkers. *Figure 10* shows an adaptation of a widely accepted, albeit simplified hypothetical model of these time courses based on recent studies and reviews. Sigmoid rather than linear fitting has been shown to represent the actual time course of biomarkers more accurately [303].

A modified hypothetical time course model is proposed in "Conclusions & Outlook" at the end of this summary.

Treatment

AD is to date still an incurable disease. The only commercially available treatment options involve today symptomatic treatment with AChEI such as donepezil, rivastigmine and galantamine. These are licensed for mild-to-moderate AD and have been proven to improve cognition, function, and global clinical outcome in several controlled randomised trials [304, 305]. PET studies have furthermore shown beneficial effects on measures such as blood flow or glucose metabolism [147, 306, 307]. Except for AChEI, only memaninte, an N-Methyl-D-aspartic acid (NMDA)-receptor antagonist is so far licensed for treatment of moderate-to-severe AD. This drug has also shown to have cognitive and neuropsychiatric benefits in trials [308].

The amyloid hypothesis has been agenda setting in the development of several treatment strategies. Most attempts to attack potential mediators of amyloid generation have failed, which is one of the reasons the hypothesis has been questioned [36, 308]. But also most other approaches have failed so far (for a concise review of reported and ongoing trials, see [308]). The general understanding of AD pathology has not been sufficient to define a suitable treatment target, yet.

Aims

The general aim of this thesis was to study eoFAD and sAD at different pre-symptomatic and symptomatic disease stages by using multiple PET tracers, alone or in combinations, in relationship to other biomarkers of AD and/or *post-mortem* pathology. The specific aims of each study were:

Paper I

- To determine whether an abnormal pattern of cerebral glucose metabolism as measured by FDG PET could be identified in young pre-symptomatic carriers of a His163Tyr *PSEN1* mutation on two subsequent occasions.
- To assess the time course of glucose metabolism from pre-symptomatic to symptomatic stages, as well as the pattern of amyloid deposition by means of PIB PET retention in one of the mutation carriers.

Paper II

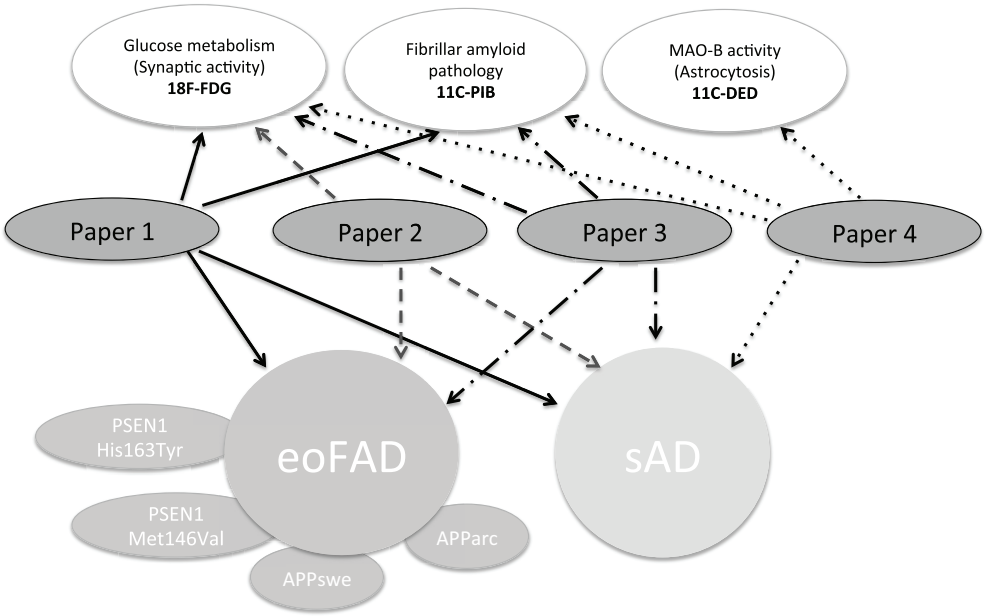
- To examine the time course of glucose metabolism in carriers of the Met146Val *PSEN1* mutation in relation to neuropsychological performance.
- To explore the relationship of regional patterns of impaired glucose metabolism with *post-mortem* neuropathology.

Paper III

- To investigate the particular amyloid pathology of the Arctic *APP* mutation *in vivo* with PIB PET in relation to CSF biomarkers, glucose metabolism, and MRI measures of atrophy in comparison with other eoFAD mutations and sAD.

Paper IV

- To determine if there is an increase of MAO-B by means of DED PET binding as a measure for astrocytosis in sporadic MCI and AD patients compared to healthy controls and if there is a regional relationship between DED, PIB, and FDG PET measures.



Subjects & Methods

Ethics approval

All studies were conducted according to the Declaration of Helsinki and subsequent revisions and approved by the Regional Ethics Committee of Stockholm, Sweden and the Isotope Committee of Uppsala University, Uppsala, Sweden.

Subjects

The participants of all four studies were recruited from the Department of Geriatric Medicine at the Karolinska University Hospital Huddinge. The clinical routine examinations generally involved physical examination, evaluation of neurological and psychiatric status, blood and CSF sampling, *ApoE* genotyping, MRI, MMSE testing, and detailed neuropsychological assessment. All subjects or their caregivers provided written informed consent to participate in the study.

AD was clinically diagnosed according to the NINCDS-ADRDA criteria [104], often confirmed by biomarker results, MCI was diagnosed according to the criteria outlined by Petersen et al. [123] (see "Clinical diagnosis").

Neuropsychological assessment

The battery of routine neuropsychological tests that all subjects underwent included measures of the following cognitive domains:

- Global cognition (MMSE [107], Full-Scale IQ (FSIQ)*)
- Episodic memory (Rey-Auditory Verbal Learning Test (RAVLT) learning and recall, Rey-Osterieth Complex Figure Test recall [120])
- Short-term memory (Digit Span*, Corsi Span)
- Visuospatial abilities (Block Design*, Rey-Osterieth Complex Figure Test copy)
- Verbal abilities (Information*, Similiarities*)
- Executive function and attention (Digit Symbol*, Trail Making Test A and B)

(* Wechsler Adult Intelligence Scale - Revised (WAIS-R) [119])

PET scanning

All PET examinations were performed using the following scanners:

- GEMS 2048-15 and GEMS 4096-15 scanners (General Electric Medical Systems, Wisconsin, USA) featuring eight rings of bismuth germanate (BGO) crystal detectors which enable the acquisition of 15 contiguous image plans over a field of view (FOV) of 104 mm (resolution x/y/z = 5.8/5.8/6.5 mm) *(Papers I and II)*.
- EXACT HR+ scanner (Siemens/CTI) with 32 rings of BGO detectors, providing 63 contiguous image planes over a FOV of 155 mm (resolution 4.4/4.4/2.46 mm) *(Papers I, III, and IV)*.
- Discovery ST PET/CT scanner (General Electric Medical Systems, Wisconsin, USA) with 24 BGO detector rings, providing 47 image planes over a FOV of 157 mm (resolution 5.7/5.7/3.27 mm) *(Paper III)*.

The scans were conducted under resting conditions in a darkened room with low ambient noise and with the patients' heads supported by quick-setting foam. Scanning was performed in three-dimensional mode, corrected for tissue attenuation, radiation scatter, and random coincidences and reconstructed by filtered backprojection using a Hanning filter. Following reconstruction, the resulting dynamic time series were corrected for motion.

Paper I

Subjects and study design

A group of six pre-symptomatic carriers of a His163Tyr *PSEN1* eoFAD mutation underwent FDG PET examinations on two occasions over a period of two years. The data was compared with data from a group of 23 healthy controls who did not carry an eoFAD mutation.

One mutation carrier was followed up longitudinally with FDG PET scans over a period of 12 years from baseline, following the subject from pre-symptomatic to symptomatic disease stages. On the last occasion, a PIB PET scan was performed and evaluated in comparison with data from a group of 27 sAD patients.

	Pre-symptomatic MC (*n*=6)	**HC (*n*=23)**
Gender (F/M)	-/6	9/14
Age (y) ± SD	34.2 ± 7.4	46.0 ± 12.2
Distance from expect age of disease onset (y)	-20	
Education (y) ± SD	11.0 ± 1.3	10.8 ± 2.5
MMSE ± SD	29.2 ± 1.3	29.2 ± 0.8
APOE ε4+	3	4

Table 6. Demographics at baseline. MC: *PSEN1* His163Tyr mutation carriers; HC: Healthy controls.

PET data acquisition and processing

FDG PET scans were performed in all subjects at baseline and two years later. FDG uptake in the brain was monitored for 60 min. FDG and glucose plasma levels were measured in venous blood samples collected during scan time from the back of the hand. Modelling according to the Patlak graphical procedure [309] was used to calculate CMRglc, expressed in μmol/min/100g, and to create parametric images. Resulting values were divided by values measured in the pons to correct for inter-subject variations, since the pons has been reported to be amongst the least affected regions in AD [310].

One MC was examined with additional FDG PET scans 10 and 12 years after baseline and a PIB PET scan on the last occasion. PIB retention was scanned over 60 minutes. Mean uptake values were obtained from a 40-60 min summation image and further transformed into SUVs. The pons was used as a reference region (instead of the

cerebellar cortex) to create SUVR ratios, as cerebellar presence of amyloid pathology had been reported previously [311].

PET data analysis

A set of 46 regions of interest (ROIs) was manually defined using a Scanditronix program (IDA, Images Display and Analyses GE 1994). All ROIs were paired for the right and left hemispheres, except for the pons and whole brain. Cortical ROIs were defined in the frontal, frontal association, parietal, parietotemporal, anterior cingulate, posterior cingulate, sensorimotor, primary visual, and cerebellar cortices. The ROI in the thalamus was drawn freehand with a size of 1 cm^2. At the level of the thalamus, ROIs encompassing the putamen and the caudate nucleus were defined. Two circular ROIs (1.5 cm in diameter) were defined in the pons in two slices. A ROI in the white matter was defined at the level of the centrum semiovale and a ROI including the whole brain at the level of the thalamus. VOIs were created by linking multiple ROIs in corresponding regions.

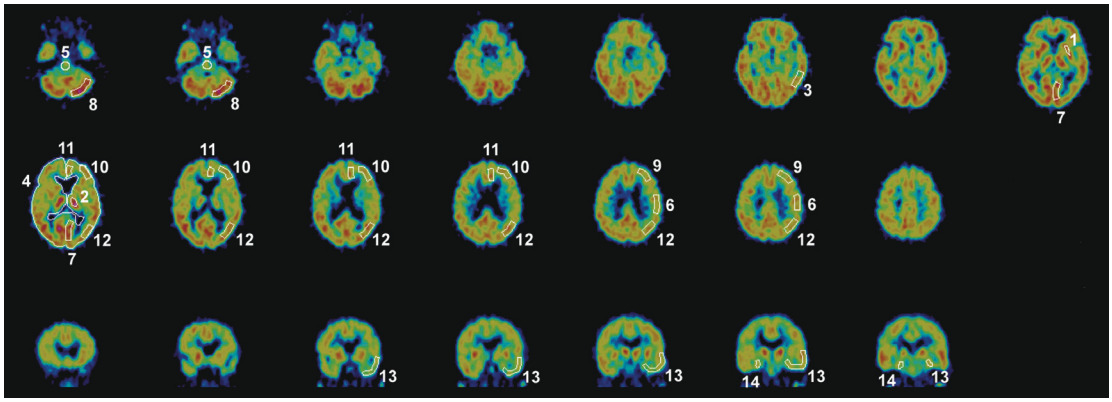


Figure 11. Location of manually outlined ROIs *(Papers I* and *II)*

Statistics

FDG PET ROI data were compared between mutation carriers and non-carriers by multivariate data analysis, namely partial least squares-discriminant analysis (PLS-DA) [312], employing SIMCA software (Umetrics AB, Umeå, Sweden). The results of the PLS-DA were visualised by plotting two components of the model against each other. Furthermore, all variables (in this case brain regions/ROIs) included in the analysis were ranked and plotted according to their importance regarding the separation of mutation carriers and non-carriers (variable of importance, VIP).

Statistical parametric mapping

Statistical parametric mapping was carried out using SPM2 (Wellcome Department of Cognitive Neurology, University College London) with Matlab 6.5 software (Mathworks Inc., Sherbon, MA). Image data were spatially normalised into the SPM2 PET template in the Montreal Neurological Institute (MNI) space. A mask was created that included only those voxels present in all scans and applied to all individual images prior to spatial smoothing with a Gaussian kernel at 12 mm (FWHM). Global normalisation was performed by proportional scaling (global value 50ml/min/100g, grey matter threshold 0.8). Differences between groups were analysed by two-sample *t*-test. The contrast was defined to test the hypothesis that a decrease in FDG might occur in the MCs. We investigated hypometabolic brain areas of $k>50$ voxels at a height threshold of $p = 0.001$ (uncorrected). For visualisation of the *t* score statistics (SPM T map), the accordant voxels were projected onto the MRI template provided by SPM2. Local maxima were labelled using the Anatomical Automatic Labeling (AAL) atlas [313].

Paper II

Subjects and study design

Four siblings, whereof two carried a Met146Val *PSEN1* eoFAD mutation, underwent longitudinal FDG PET scanning over a period of two and four years, respectively. The longitudinal FDG PET data was put in relationship to neuropsychological test data and FDG PET data from a group of 23 healthy controls and a group of 27 sAD patients.

Furthermore, neuropathology was assessed *post-mortem* in the brains of the mutation carriers, compared with data from a reference group of 249 sAD patients and related to regional impairment of glucose metabolism on the last scan occasion before death.

PET data acquisition, processing, and analysis

All subjects underwent FDG PET scanning on one or several occasions. The PET examinations and ROI analysis of FDG PET data were performed in accordance with the methodology described in *Paper I*. MC-1 received two, MC-2 six, and NC-1 and -2 five consecutive FDG PET scans. The first scan of MC2, however, had to be excluded due to technical issues.

	MC-1	MC-2	NC-1	NC-2	HC (*n*=23)	sAD (*n*=27)
Gender (F/M)	F	F	F	M	9/14	13/14
Age (y) ± SD	41.5	40.9	36.3	26.4	46.0 ± 12.2	66.2 ± 9.2
Distance from age of disease onset (y)	+4.5	+1.9				
Education (y) ± SD	9	9	11	11	10.7 ± 2.7	12.1 ± 3.4
Longitudinal FDG PET scans	2	6	5	5		
MMSE ± SD	14	24	30	30	29.0 ± 0.9	22.4 ± 5.2
APOE ε4	+	+	-	-	4	17

Table 7. Demographics at baseline. MC: Met146Val mutation carriers; NC: Mutation non-carriers; HC: Healthy controls; sAD: Sporadic AD patients.

Post-mortem tissue analysis

Bielschowsky silver impregnation was used to stain AD neuropathology in the brains of the mutation carriers, which had been handled and dissected according to routine protocols for the Huddinge Brain Bank [314]. Neurofibrillary tangle (NFT) and neuritic plaque (NP) pathology was quantified throughout the cortical thickness of several brain regions using a stereological analysis system.

Statistics

Repeated Measures Analysis of Variance, followed by Bonferroni *post-hoc* testing, was used to test if a statistically significant time effect could be observed in the longitudinal FDG PET data from MC-2. Correlations of FDG PET data with neuropsychological test results and quantitative measures of neuropathology were performed by Spearman's Rank correlation.

Paper III

Subjects and study design

Two carriers of the Arctic *APP* (*APP*arc) eoFAD mutation and five related non-carriers, as well as one carrier of the above mentioned *PSEN1* His163Tyr mutation, one carrier of the Swedish *APP* (*APP*swe) mutation, seven sAD patients and seven healthy controls were examined with PIB and FDG PET, MRI, and CSF measures of $A\beta_{1\text{-}42}$, t-tau and p-tau.

PET data acquisition and processing

PIB retention was scanned dynamically over 60 min resulting in 24 time frames and FDG uptake over 45 min resulting in 21 frames. 40-60 min PIB summation images and 30-45 min FDG summation images were created for subsequent analysis.

	APParc-1	APParc-2	APParc NC (*n*=5)	PSEN1-1	APPswe-1	sAD (*n*=7)	HC (*n*=7)
Gender (F/M)		1/6		M	M	3/4	2/5
Age (y) ± SD		56.1 ± 12.8		58		64.1 ± 6.1	59.7 ± 9.5
Education (y) ± SD		12.0 ± 3.4					
Distance from expect age of disease onset (y)	+3	-3		+4	-1		
MMSE ± SD	11	30	28.8 ± 1.3	11	26	24.4 ± 5.7	24.4 ± 5.7
APOE ε4+	-	-	1	+	+	5	2

Table 8. Demographics. NC: Mutation non-carriers; sAD: Sporadic AD; HC: Healthy controls. Values for all *APP*arc family members are displayed as mean ± SD and age for APPswe-1 is not shown for reasons of confidentiality.

MRI data acquisition and analysis

All participants underwent structural T1 MRI scanning at 3T. Cortical thickness and hippocampal volume measurements were carried out using a processing pipeline included in the Freesurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu).

PET data analysis

A modified version of a digital probabilistic atlas [315] was used in each individual's original MRI space to extract regional PIB and FDG PET data, resulting in 29 VOIs *(Figure 12)*.

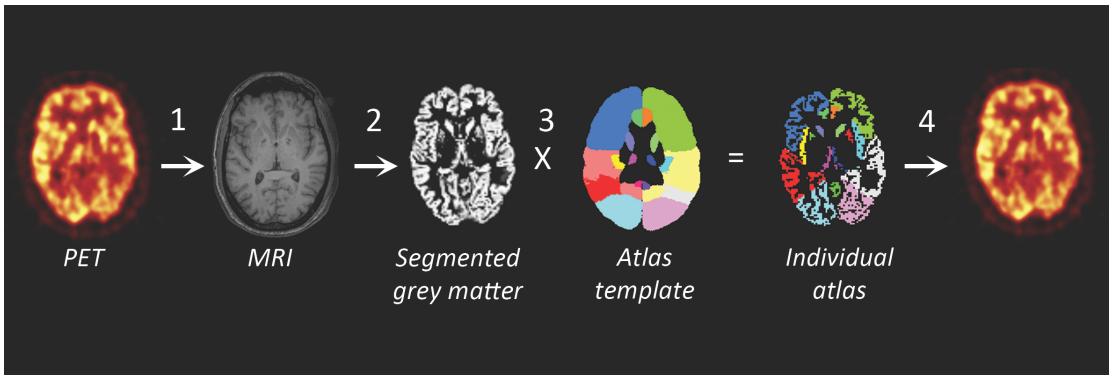


Figure 12. Atlas-based ROI analysis. 1. Co-registration of PET and MRI images. 2. Segmentation of MRI image. 3. Multiplication of a binary map created from the segmented grey matter MRI with the predefined atlas volume. 4. Application of the resulting individual atlas onto the co-registered PET image and extraction of regional values.

Statistics

For group comparisons, the Mann-Whitney U Test was applied. For individual subject comparisons, z-scores were created for each modality based on the values from the HC.

Paper IV

Subjects and study design

Eight MCI and seven sAD patients were scanned with FDG, PIB, and DED PET. The MCI patients were divided into MCI PIB+ and MCI PIB- according to their levels in PIB retention. The DED PET data were compared with data from a group of 14 healthy controls.

	HC (*n*=14)	MCI PIB- (*n*=3)	MCI PIB+ (*n*=5)	sAD (*n*=7)
Gender (F/M)	9/5	1/2	3/2	3/4
Age (y) ± SD	64.7 ± 3.6	65.3 ± 6.6	61.0 ± 8.3	65.0 ± 6.3
Education (y) ± SD				
MMSE ± SD		27.7 ± 2.3	27.4 ± 2.3	24.4 ± 5.7
APOE ε4+		1	3	5

Table 9. Demographics. HC: Healthy controls; MCI: Mild cognitive impairment patients; sAD: Sporadic AD patients; PIB +: Sum/pons values > 1.41.

PET examinations, processing, and analysis

All FDG and PIB PET examinations were performed and the images processed and analysed as in *Paper III* except for that all analysis were performed in DED PET native space to preserve the DED scans integrity. DED PET acquisition was conducted over 60 min (19 time frames). Dynamic DED uptake data was modelled between 20 to 60 min according to a modified reference Patlak approach [316], using the cerebellar grey matter corrected for net tracer accumulation as reference region. This modelling produced two measurements: Intercept (initial tracer distribution volume) and slope (binding of DED to MAO-B) *(Figure 13)*.

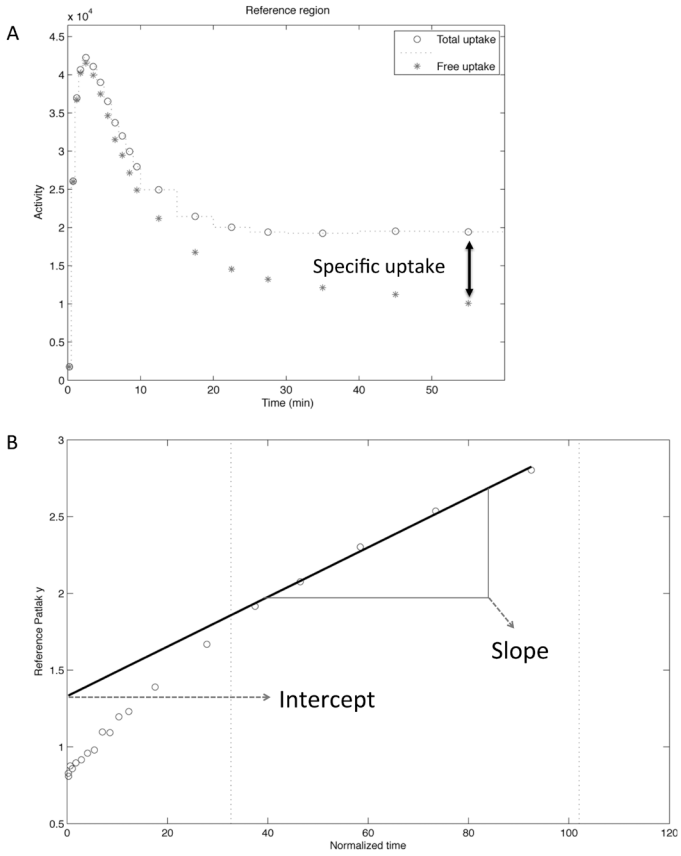


Figure 13. Modelling of DED PET data. (A) Time activity curve for the cerebellar reference region before and after correction for specific tracer binding using the smallest fixed correction factor that would still lead to linearisation in the Patlak plot (B).

Statistics

Analysis of Variance (ANOVA) with Fisher's Least Significant Difference (LSD) *post-hoc* testing was employed to test for significant group differences. Relationships between the different PET tracers were tested by Pearson's correlation with and without Bonferroni correction for multiple comparisons.

Results & Discussion

Early thalamic glucose hypometabolism in *PSEN1* His163Tyr mutation carriers

The pattern of cerebral glucose hypometabolism in sAD and MCI is well described, commonly involving posterior cingulate, parietotemporal, and hippocampal impairment, with other regions being affected at different disease stages (see "PET in AD: Glucose metabolism"). However, only very few studies have assessed this pattern in carriers of eoFAD mutations. Early studies have shown focally decreased glucose metabolism in the parietotemporal cortex against a background of global impairment and mild cortical atrophy as measured with MRI in pre-symptomatic *PSEN1* mutation carriers when compared with age-matched healthy controls [191, 192]. These subjects were on average examined 2-3 years prior to their expected age of onset. In a longitudinal study, glucose metabolism in temporal cortical regions in pre-symptomatic *APP*swe mutation carriers was assessed and it was found to be decreased in these regions several years before disease onset, exceeding MRI measures of atrophy [138]. More recently, another study showed that hypometabolism mainly in the posterior cingulate, entorhinal, inferior parietal and superior temporal cortices as well as in the hippocampus and the whole brain, preceding atrophy in pre-symptomatic carriers of *PSEN1* carriers approximately 13 years before disease onset [137]. Finally, one study combined FDG with PIB PET examinations in seven pre-symptomatic and symptomatic *PSEN1* and one *APP* mutation carriers, observing no consistent pattern of hypometabolism in early pre-symptomatic stages, while PIB retention seemed to start in the striata of these individuals [227].

We examined six pre-symptomatic carriers of a *PSEN1* His163Tyr mutation on two occasions, on average 20 and 18 years before expected disease onset, respectively, in comparison with 23 age-matched non-carriers *(Paper I)*. SPM analysis revealed lower glucose metabolism in the right thalamus of the mutation carriers, first by trend, and at follow-up statistically significant (baseline: n.s., follow-up: p=0.028 corrected at cluster level; p=0.034 corrected at voxel level) *(Figure 14)*. PLS-DA furthermore showed significant separation of mutation carriers from non-carriers, reaching 83% sensitivity and 100% specificity at baseline and 100% sensitivity and specificity at follow-up, using all FDG PET ROIs as variables. The region contributing most to the separation between the groups was the right thalamus on both occasions, with other regions such as the left thalamus, right parietotemporal, right posterior cingulate, and right temporal cortex

ranking thereafter. The thalamus has previously been reported to be hypometabolic in pre-symptomatic and symptomatic eoFAD and sAD [194, 227, 317] and to show decreased activation in response to a memory paradigm in a large fMRI study in asymptomatic individuals at risk for familial AD [318]. Similar to our findings, clear lateralisation of hypometabolic patterns has also been observed before in early stages of AD, potentially reflecting neuroplastic compensatory mechanisms [171, 173].

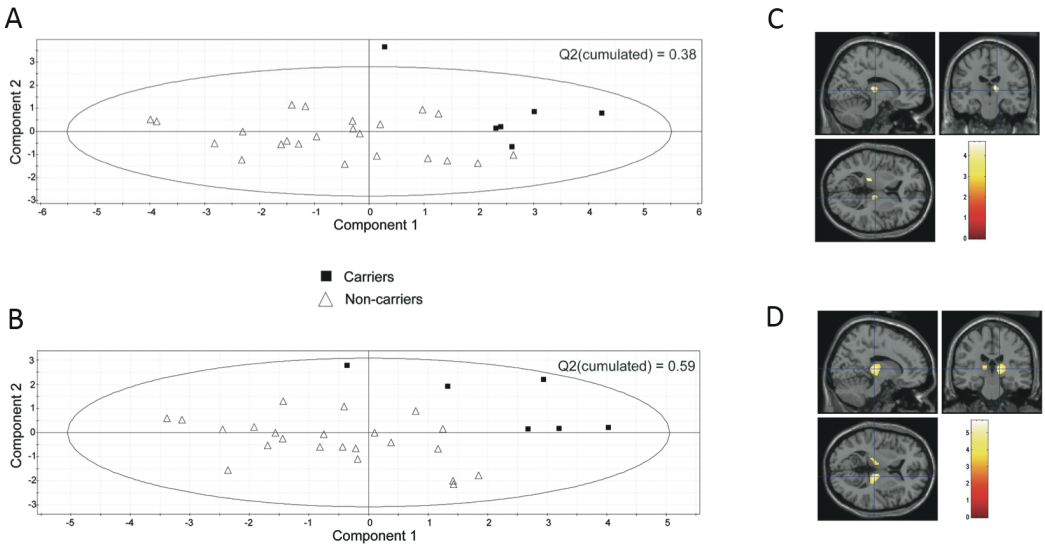


Figure 14. Results from PLS-DA and SPM analyses at baseline (A, C) and after two years (B, D) showing separation of mutation carriers and non-carriers by FDG PET (A, B) and the thalamus differing between the groups.

The thalamus is likely to be involved in early pathological changes in the development of AD. Thalamic connections are pivotal constituents of networks involved in memory processing that include brain regions such as the posterior cingulate cortex, the hippocampus, and cortical association areas, which commonly reveal decreased glucose metabolism in later disease stages [319-322].

Our findings suggest that impairment of glucose metabolism started in the thalamus of our mutation carriers. Methodological considerations when interpreting the data should, however, be taken into account. Analysis of regional glucose metabolism with FDG PET without correction for partial volume effects might be compromised in smaller structures, for example, by proximity to the CSF, as is the case with the thalamus. Since our data set was relatively small and only comprised carriers of one specific eoFAD mutation, general conclusions regarding both eoFAD and sAD should be drawn with caution.

Time course of glucose metabolism and cognitive decline in *PSEN1* His163Tyr and Met146Val mutation carriers

Glucose metabolism has not only been shown to be decreased in early pre-symptomatic stages of AD, it is also known to be closely related to cognitive decline and disease severity [179]. We followed one of the above-mentioned carriers of a His163Tyr mutation from age 46 to 57 – clinical diagnosis of AD was made at age 55 – and two carriers of a Met146Val mutation, one from age 41 to 43 (diagnosed with AD at age 39) and one from age 41 to 45 (diagnosed with AD at age 40), respectively *(Papers I and II)*.

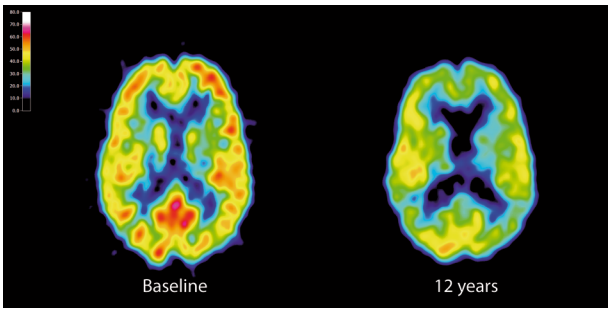


Figure 15. Decline in glucose metabolism in a *PSEN1* H163 mutation carrier.

Longitudinal FDG PET and neuropsychological test data were compared with data from 23 coeval healthy controls and 27 sAD patients who were on average approximately 25 years older than the Met146Val mutation carriers at baseline. All mutation carriers showed global decrease of glucose metabolism when compared with the controls, but the regional patterns differed slightly between pre-symptomatic and symptomatic stages.

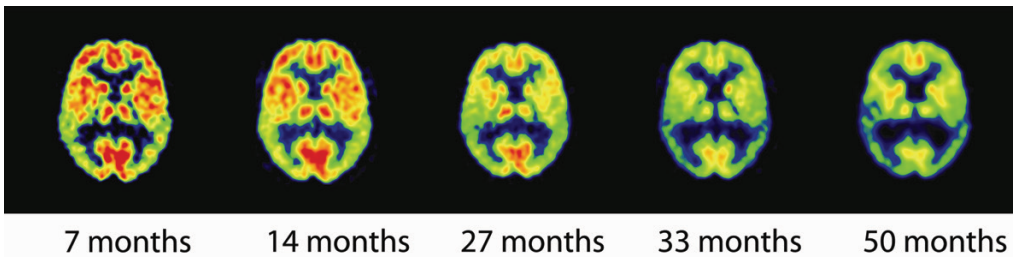


Figure 16. Decline in glucose metabolism in a *PSEN1* M146V mutation carrier.

In the His163Tyr carrier, the most prominent early changes, besides the previously mentioned thalamic impairment, occurred in the posterior and anterior cingulate and frontal cortex, whereas parietal and temporal cortical as well as striatal metabolism decreased in later stages *(Figure 15)*. The most pronounced decrease over time was

observed in the posterior cingulate cortex. Cognition was preserved until shortly before AD was diagnosed, when test results for episodic memory were deviant. Two years after the diagnosis, almost all cognitive domains were clearly impaired. A strong correlation was found between decline in posterior cingulate glucose metabolism and cognitive test scores for attention and cognitive speed (Digit Symbol Test, $p<0.005$) *(Paper I)*.

Despite their young age and relatively short disease duration, both Met146Val mutation carriers had clearly decreased levels of glucose metabolism, globally, generally comparable with levels in the much older sAD patients, and significantly deviant cognitive performance. Here, decline was most prominent in the posterior cingulate, parietal and parietotemporal cortices, while frontal and temporal cortical as well as subcortical areas were somewhat more preserved over time. Global metabolic decline was statistically significant over time (Repeated Measure ANOVA corrected for multiple comparisons, $\alpha=0.05$). The decline in global cognition correlated well mainly with decrease in CMRglc in the bilateral posterior cingulate ($p<0.001$) but also in the right parietotemporal cortex ($p=0.05$).

The regional pattern of decline in cerebral glucose metabolism observed in our studies fits well with what has been reported earlier in studies of individuals at risk for and suffering from eoFAD, MCI, and sAD [137, 180-183, 191, 192, 323]. In particular, glucose metabolism in the posterior cingulate cortex has been suggested to be a common reliable marker and predictor for cognitive decline from pre-symptomatic to early and late disease stages, whereas other typically affected brain regions might become impaired first at later symptomatic stages.

Relationship of regional glucose metabolism and post-mortem pathology in *PSEN1* Met146Val mutation carriers

The most characteristic *post-mortem* findings in the AD brain are neuritic plaques (NPs) and neurofibrillary tangles (NFTs) (see "Pathology and pathogenesis"). After having mapped the time course of glucose metabolism in *PSEN1* Met146Val mutation carriers as described above, we aimed to explore the relationship between neuropathological hallmarks of AD and *ante-mortem* decreases in glucose metabolism in the same individuals *(Paper II)*. The brains of the two aforementioned mutation carriers were subject to *post-mortem* examinations in terms of quantification of NP and NFT pathology in reference to a large data set of 249 sAD patients that were classified as Braak stage VI [324] *(Paper II)*.

Macroscopically, cortical thickness was significantly less in the mutation carriers than in the sAD patients. Microscopically, vast amounts of NPs, including mainly diffuse plaques in the putamen, and NFTs were found in almost all examined brain regions, exceeding the numbers seen in the sAD patients by far. NFTs were absent in the cerebellar cortex and the putamen in both eoFAD and sAD patients.

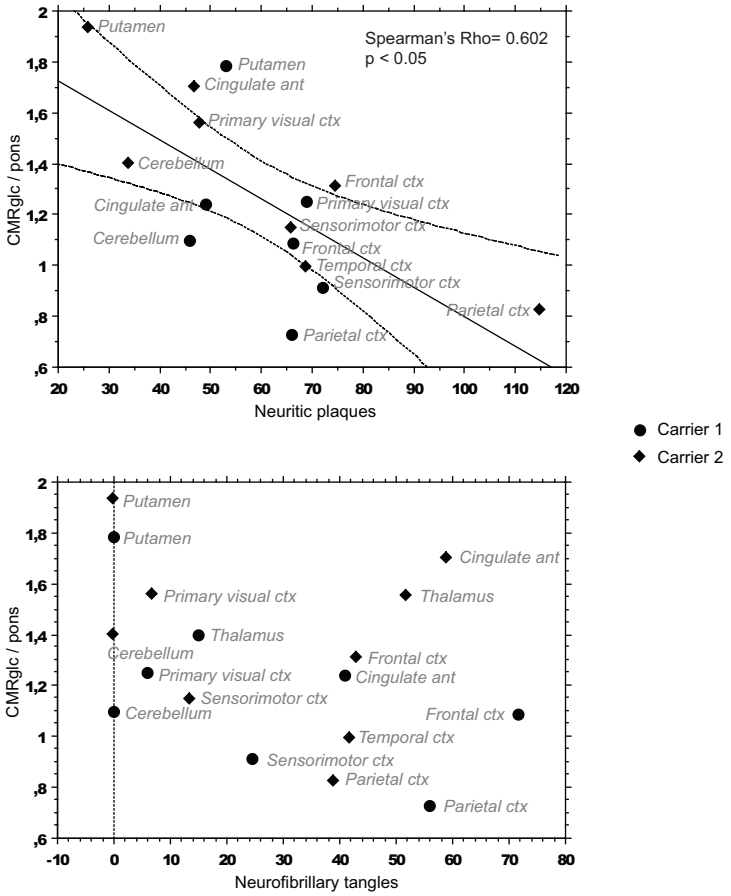


Figure 17. Correlation between regional glucose metabolism and regional amounts of neuritic plaques and neurofibrillary tangles in two *PSEN1* M146V mutation carriers.

The Met146Val mutation thus appears to be an eminently devastating eoFAD mutation, given the very early mean age of disease onset of about 38 years, the rapid decline in glucose metabolism (as described before), and the neuropathological picture presented here. *APP*swe mutation carriers with comparable disease duration had previously been shown to have comparable patterns of regional neuropathology to those in sAD patients [325], suggesting that *PSEN1* mutations, or at least the one studied at hand, might cause earlier and more severe AD than the *APP*swe mutation.

CMRglc is commonly referred to as a marker for synaptic activity, involving metabolic processes in both neurons and astroglia (see "Glucose metabolism") and a

relationship with neuropathology is therefore likely. While both CMRglc and tau pathology correlate well with disease progression [32, 177] and thus with each other during disease development, this seems not to be the case when end-stage pathology is reached. In fact, a significant negative Spearman's Rank correlation was observed in the mutation carriers between levels of right hemisphere regional CMRglc (rCMRglc) of the last *ante-mortem* FDG PET scan and the amount of NPs in the respective brain regions (n=15; Rho=−0.602; p<0.05), but not between rCMRglc and counts of NFTs *(Figure 17)*. Several previous studies have reported similar lacking or poor correlations between cerebral tau pathology or CSF levels of tau and regional rCMRglc [326-329], whereof one showed, similar to our findings a strong relationship between rCMRglc and senile plaque load in one subject [329].

Of note is that NFT pathology, consistent with the above-mentioned studies, was highly abundant in the hippocampus. This region was not included in the set of ROIs for FDG PET analysis. Moreover, there was a considerable time gap of five years between the last FDG PET scans and *post-mortem* examinations, hampering confident interpretation of our findings.

PIB retention in *APP*arc mutation carriers in relationship to AD biomarkers compared with other eoFAD mutations and sAD

Among the eoFAD mutations, those located within the sequence encoding Aβ seem to have an exceptional position. Substitution or deletion of merely one base results in entirely different phenotypes and disease types. Two of these, the Arctic *APP* (*APP*arc) and the E693Δ mutation, have been reported to favour Aβ oligomer and/or protofibril over fibril formation and to still cause a typical clinical picture of AD [95, 97, 330]. A *post-mortem* study in *APP*arc mutation carriers who had suffered from Alzheimer-like dementia demonstrated ring-formed plaques without an Aβ core that stained negative for Congo red but positive for silver impregnation and C-terminal A$\beta_{40/42}$ antibodies [98].

We were interested in how this particular Aβ pathology would picture *in vivo* in terms of PIB PET retention in the context of other established biomarkers of AD and we performed as part of a larger longitudinal multi-tracer study on eoFAD and sAD the first *in vivo* examination of seven members, of whom two were mutation carriers, of a family harbouring the *APP*arc mutation. For comparison, one carrier of the *PSEN1* His163Tyr

mutation, one carrier of the *APP*swe mutation, as well as seven healthy controls (HCs) and seven sAD patients were included in the study. One *APP*arc (APParc-1) and the His163Tyr mutation carrier (PSEN-1) had been diagnosed with AD, the subject with the *APP*swe mutation (APPswe-1) with MCI, and the second *APP*arc mutation carrier (APParc-2) reported occasional subjective cognitive impairment but had not received a clinical diagnosis.

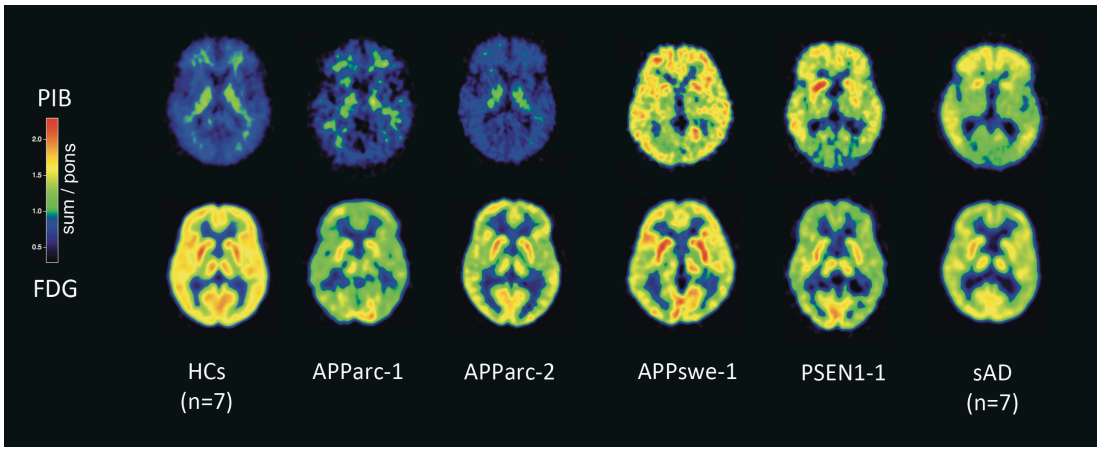


Figure 18. PIB and FDG PET scans of seven healthy controls, the two carriers of the *APP*arc mutation, one *PSEN1* H163Y mutation carrier, one *APP*swe mutation carrier, and seven sAD patients. Of note is the lack of PIB retention in the *APP*arc mutation carriers. HCs: Healthy controls; sAD: Sporadic AD.

Both *APP*arc mutation carriers presented with "negative" PIB PET scans, reflecting very low grey matter retention, comparable with the scans of their related mutation non-carriers and the healthy controls *(Figure 18)*. This was despite otherwise clearly pathological CSF measures of $A\beta_{1\text{-}42}$, t-tau, and p-tau *(Figure 19)*. APParc-1 furthermore showed globally decreased glucose metabolism and significant atrophy in the hippocampus and entorhinal cortex in comparison with the HCs. APParc-2 revealed focally decreased glucose metabolism in posterior cingulate, temporal, and parahippocampal cortices, as well as in hippocampus, but no atrophy. PSEN-1 had been examined with PIB PET one year before *(Paper 1)*, demonstrating high cortical PIB retention and especially elevated levels in the striatum. This pattern was basically unchanged on this occasion, with striatal PIB retention exceeding levels in sAD patients. CSF measures, global glucose metabolism, and hippocampal volume were significantly lower than in HCs. PIB retention was globally high also in APPswe-1 with levels in the striatum, thalamus, and hippocampus being higher than in sAD patients. CSF levels of $A\beta_{1\text{-}42}$ and p-tau were pathological whereas glucose metabolism and brain structure were preserved.

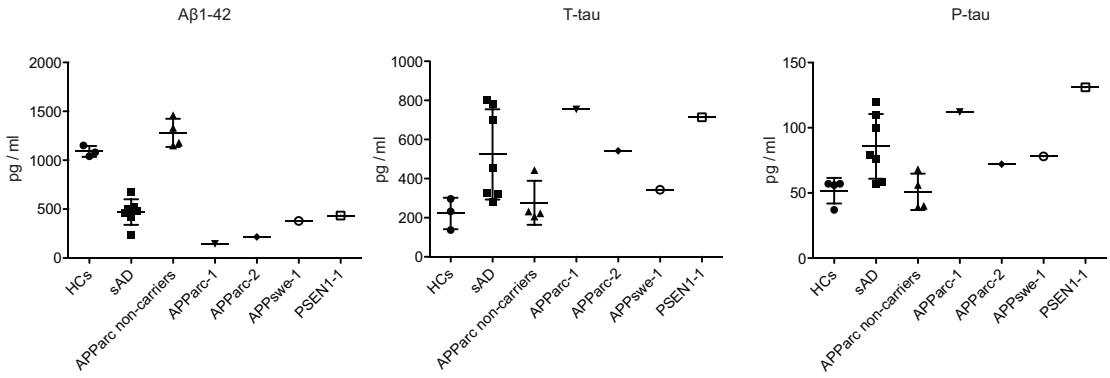


Figure 19. CSF data of all study participants. All eoFAD mutation carriers, including the two *APP*arc mutation carriers, showed pathological levels of A$\beta_{1\text{-}42}$ and tau in CSF.

Elevated cortical levels of PIB retention have been reported in over 90% of patients with a clinical diagnosis of AD and are furthermore consistently found in early symptomatic and pre-symptomatic disease stages [297, 331]. Rare cases are known where a clinical picture of AD coincided with a lack of PIB binding, suggesting that a certain threshold of binding must be reached for detection with PET [222]. Patients with eoFAD also commonly show high PIB retention, often characterised by focally increased striatal binding already at early pre-symptomatic stages [226, 227]. PIB retention is also well known to be inversely related to CSF levels of A$\beta_{1\text{-}42}$ [332, 333]. PIB has been proven to be a specific marker for extracellular and intravascular fibrillar Aβ structures, binding to the β-pleated sheet conformation of the Aβ fibrils [207, 208, 334, 335]. The plaques found in a *post-mortem* examination of *APP*arc mutation carriers did not stain for Congo red or thioflavine-S, illustrating a lack of β-pleated sheet fibrils of Aβ [98].

Our findings of negative PIB scans in the presence of low levels of CSF A$\beta_{1\text{-}42}$ in *APP*arc mutation carriers, who also showed characteristics typical of AD in other biomarkers, suggest that forms of Aβ other than mature β-sheet fibrillar forms can cause AD-type dementia. Since *APP*arc promotes protofibril formation at high levels [97], these forms are likely to underlie the clinical picture of AD in our mutation carriers. This is supported by the recent discovery of the E693Δ mutation in the same amino acid positions as the *APP*arc mutation, which causes recessively inherited AD type dementia by way of enhanced formation of Aβ oligomers [95]. Analogous to our results, PIB PET retention in symptomatic carriers of this mutation was very low while CSF levels of A$\beta_{1\text{-}42}$ were pathologically low.

The pathological effects of Aβ oligomers and protofibrils have so far only been studied *in vitro* [39-45]. Our study might provide a link between these studies and clinical disease. Furthermore, it is important to mention that CSF Aβ_{1-42} and PIB retention in AD must not necessarily be inversely related, although this might be the case in the majority of sAD cases, which has already led to approaches to transform CSF Aβ_{1-42} levels into PIB retention [336].

Evidence for astrocytosis in prodromal AD

Neuroinflammation is a prominent feature in AD, mediated predominantly by microglia and astrocytes. Activated astrocytes have been found to gather predominantly around Aβ plaques and to play an important role in Aβ plaque formation and Aβ clearance [77, 79-84]. The time course of astrocyte activation in the AD disease process is thereby still unknown. In order to explore the relationship between fibrillar Aβ pathology and astrocytosis and the underlying time course, we performed a multi-tracer PET study in MCI and mild AD patients in comparison with healthy controls, using ^{11}C-L-deuterium-deprenyl (DED) (see "PET in AD: Neuroinflammation"), PIB, and FDG PET *(Paper IV)*.

The modified reference Patlak model we used for kinetic modelling of the DED data generates an intercept (tracer delivery) and a slope value (tracer binding). We found that the PIB+ MCI patients had the highest DED slope values in all examined cortical brain regions, somewhat less so in subcortical regions, whereas the AD patients presented with slope values generally comparable with those observed in the controls. Initial ANOVA, involving the undivided MCI patient group, revealed significant group effects in the frontal and parietal cortices. The intercept values followed the pattern HC>MCI>AD but did not differ significantly between the groups. Since MCI patients who show elevated PIB retention are commonly more likely to progress to AD than those with low levels [220, 337], we divided the MCI patients into PIB positive (+) and PIB negative (-), applying a previously defined cut-off. Subsequent statistics then revealed significant group effects of DED slope values now in the right occipital cortex and right hippocampus. The PIB+ MCI patients showed FDG and PIB PET measures comparable with those among the AD patients and were in an intermediate state between the PIB- MCI and the AD patients as regards cognitive test results and CSF measures. They could thus be regarded as cases of prodromal AD.

Our finding of increased DED binding in MCI patients who are likely to develop AD suggests that enhanced MAO-B activity as a measure of astrocytosis is an early phenomenon in the disease process. This concept is supported by several observations. A recent autoradiography study using ^{11}C-L-deprenyl in the brains of AD patients with different Braak stages found a distinct pattern of decreased binding with increased Braak staging with early stages (I-III) showing highest overall binding. Among the multitude of functions that astrocytes exert during neuroinflammation are many associated with Aβ. Hence, extracellular Aβ deposition has been shown to trigger astrogliosis [338] and astrocytes participate in the formation of Aβ plaques and in Aβ clearance [82-84, 339], both of which are processes likely to be enhanced at an early disease stage phenotypically represented by MCI. The question then arises is if astrocytosis should be regarded as a product or a producer of Aβ. MCI is also a state of early neurodegeneration as visualised, for example, by many MRI studies on brain atrophy and damaged or degenerating neurons are strong initiators of reactive astrocytosis [338]. Moreover, both neurons and astrocytes have been reported to undergo apoptosis in AD [340], possibly implying both less need for astrocytic support of neurons and astrocyte degeneration as an explanation for the decreased DED slope values observed in our AD patients.

Given the close relationship of astrocytosis and Aβ as well as the role that astrocytes are suggested to play in glucose metabolism, a correlation between DED binding and PIB and/or FDG signal could have been expected. However, no regional relationship was observed between any of the three tracers. This is in agreement with results of a recent study from our group that revealed a lack of relationship between *in vivo* ^{11}C-PIB and ^{3}H-PIB binding versus ^{3}H-L-deprenyl binding and *in vivo* FDG PET uptake versus ^{3}H-L-deprenyl binding at autopsy of the first-ever PIB examined patient [209]. Highest ^{3}H-L-deprenyl was thereby observed in the hippocampus. *In vivo* PIB retention and levels of *post-mortem* GFAP immunostaining did correlate, indicating that MAO-B activity is a marker for a specific, reactive type of astrocyte, while GFAP is rather unspecific. Furthermore, a recent study in an AD mouse model demonstrated astrocyte hyperactivity in a synchronised manner across a larger cortical network, amplifying focal Aβ deposition [341]. Aβ deposits catalysed thus astrocyte activation distant from the actual Aβ pathology, which fits with the observed lack of regional correlation between DED binding and PIB retention in our study.

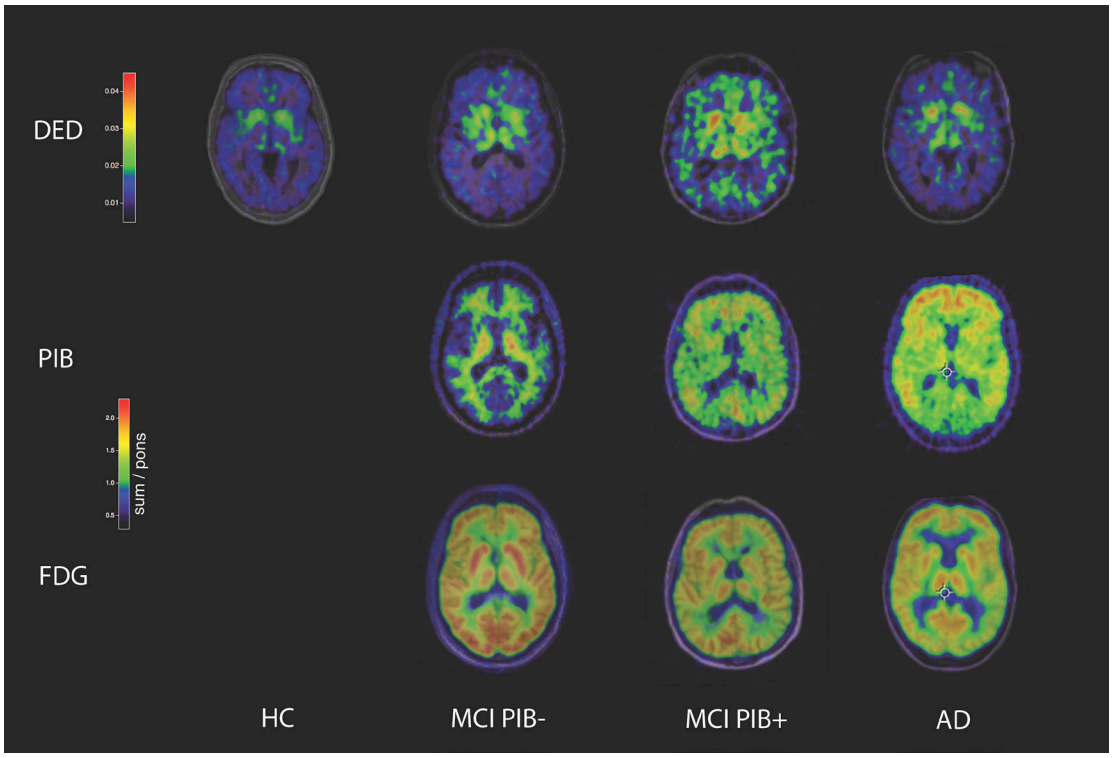


Figure 20. Representative DED slope parametric and PIB and FDG sum/pons images. The MCI PIB+ patient showed the highest diffuse cortical DED binding.

The finding of increased DED binding early in AD is intriguing. Methodological considerations should, however, be taken into account. It is not totally clear to what extent MAO-B expression equals astrocyte activation, since MAO-B has also been found in serotonergic neurons. In addition, the DED signal as observed by PET examination is known to be strongly related to blood flow. When using this tracer, kinetic modelling is thus essential to account for the blood flow component. Without arterial input data, our modified reference Patlak approach can merely be considered as an approximation, in particular with regard to the fixed correction for specific binding in the reference region.

A recently published study, unlike our results, reported significant differences in DED retention between a group of healthy controls and AD patients and a regional correlation between DED and PIB retention [254]. On closer inspection, however, it became obvious that there was actually no difference between DED slope values in controls and AD patients. Both the reported differences in tracer retention and tracer relationship were based on a slope/intercept ratio. This measure, originally proposed by the author of this thesis, was meant to account for differences in blood flow between different individuals. In fact, the intercept values in the AD patients were 15% lower than in the

controls, accounting completely for the observed differences in slope/intercept ratio. This could mean that differences in brain perfusion might have been measured rather than actual tracer binding. The scientific value of this ratio is thus yet to be established.

We are currently in the process of finalising the first cross-sectional phase of a large longitudinal multi-tracer PET study including members of families harbouring eoFAD mutations, and MCI and sAD patients. Preliminary analyses have confirmed a pattern of increased DED binding in early, pre-AD stages. Especially young pre-symptomatic eoFAD mutation carriers and, again, PIB+ MCI patients demonstrated the highest DED binding. Thorough analysis will show if there are clear temporal and spatial patterns in DED binding in eoFAD and sAD.

Concluding remarks & Outlook

The general intention of this work was to use multi-tracer PET to investigate different pathological characteristics of eoFAD and sAD at pre-symptomatic and symptomatic disease stages, in relationship to other biomarkers of AD and/or *post-mortem* pathology.

The main findings outlined following the order of presented papers are:

- A decline in glucose metabolism was shown subcortically, more specifically in the thalamus, in very early pre-symptomatic *PSEN1* eoFAD mutation carriers and extended over time into regions typically affected in sAD.
- Glucose hypometabolism preceded cognitive impairment by many years in *PSEN1* eoFAD mutation carriers.
- The regional pattern of glucose hypometabolism correlated with neuritic plaque pathology, not neurofibrillary tangles, in severe *PSEN1* eoFAD cases.
- Carriers of the *APP*arc mutation showed absence of cortical PIB retention at different disease stages although CSF measures of $A\beta_{1-42}$ and tau together with FDG PET revealed clear progressing AD pathology.
- Hippocampal and entorhinal cortical atrophy was only present in later disease stages in eoFAD mutation carriers.
- Symptomatic *PSEN1* and *APP*swe mutation carriers showed high cortical PIB retention, exceeding sAD levels predominantly in the striatum, and CSF measures of $A\beta_{1-42}$ and tau, while glucose metabolism was preserved in the *APP*swe carrier.
- MAO-B activity as a marker of astrocytosis was increased in prodromal AD whereas AD patients had MAO-B activity comparable to that in healthy controls.
- Fibrillar $A\beta$ deposition, glucose metabolism, and astrocyte activation showed no regional relationship.

Several conclusions can furthermore be drawn from this thesis:

- Astrocytosis might be most pronounced in early disease stages, according to preliminary data both in sAD and eoFAD
- A clinical phenotype of AD can be caused by other than fibrillar, β-sheet forms of $A\beta$, supposedly by protofibrillar or oligomeric species.
- CSF measures of $A\beta_{1-42}$ do not necessarily correlate with PIB retention in AD.

- FDG PET is a reliable measure of disease severity in eoFAD, from very early pre-symptomatic to terminal disease stages.
- Subjects with eoFAD mutations show great variations in age of onset, disease progression, and pathology, not necessarily compatible with sAD

At this point, the author would like to elaborate on aspects that have come to special attention during the course of this thesis work.

Amyloid

The amyloid cascade hypothesis states that Aβ sooner or later aggregates into insoluble, neuritic plaques. Its revision in turn implied that Aβ toxicity is mediated by oligomeric species; this has so far only been confirmed *in vitro*. With our PIB PET study in *APP*arc mutation carriers, we have established a connection between a mutation that enhances Aβ protofibril formation and a clinical phenotype of AD without the presence of classical neuritic plaque pathology.

Amyloid and astrocytes

The complexity of astrocytic functions and networks and their potential role in AD constitute an intriguing field of research. Astrocytes, amongst others things, are involved in memory functions, neuronal and synaptic support, and energy metabolism. Reactive astrocytes are commonly found around Aβ plaques in *post-mortem* tissue and Aβ is a major mediator of astrocyte activation. Our data suggest a lack of spatial relationship between fibrillar Aβ deposits and reactive astrocytes. However, we showed the highest DED binding in MCI patients who were likely to progress to AD. In this intermediate disease state, many factors might be able to trigger astrocyte activation. Degenerating neurons call for support, synaptic dysfunction needs increased effort, and ongoing Aβ production and accumulation triggers astrocytic activity distant from focal pathology via the astrocytic network [341]. Results from our ongoing longitudinal multi-tracer PET study will shed more light on the temporal pattern of astrocytic activation, Aβ pathology, and glucose metabolism in both eoFAD and sAD.

With regard to the use of PET in astrocyte research there are several issues to resolve. It is not completely understood to what extent MAO-B activation as visualised by

DED PET actually represents reactive astrocytes since this enzyme is also expressed elsewhere in serotonergic neurons. Combining DED PET with PET using tracers for e.g. serotonergic receptors or the S100 protein, for example, might help to elucidate this issue. The understanding of what "reactive astrocyte" actually embraces is also fragmentary. It might be possible, though completely theoretical, that astrocytes can adopt different states of activation and that increased MAO-B activation reflects just a certain activation phase or even just a certain type of astrocytes.

Of great interest would also be more investigation of the role of astrocytes in cerebral glucose metabolism, especially with regards to FDG PET scanning.

EoFAD and sAD

EoFAD is commonly proposed as a model for AD. Two recent reviews attempted to summarise characteristics of known eoFAD mutations, reporting generally good compatibility of eoFAD with sAD in terms of clinical outcome, even though many eoFAD mutations show deviant characteristics that are not directly related to dementia [99, 342]. Most of the so far examined mutations do show comparable pathology *post-mortem*, but the roads there may differ greatly and the large and growing number of different eoFAD mutations complicates drawing universal conclusions. The author is reluctant to consider the idea that eoFAD could be regarded as a general model for sAD. Pure genetic causes of AD, shifting the age at onset in certain cases down to hardly more than 30 years, strongly imply different pathogenesis in comparison with sAD. It is, however, self-evident that it remains quite feasible to study certain disease mechanisms that are common in eoFAD and sAD and the author is convinced that particularly *in vivo* studies of eoFAD are an emerging and highly important field aspect of AD research.

However, studies in eoFAD always involve an ethical dilemma. Strategies and action plans regarding how to deal with the discovery of pre-symptomatic eoFAD mutation carriers, especially among the young, are urgently needed. This concerns also potential early, pre-symptomatic treatment strategies.

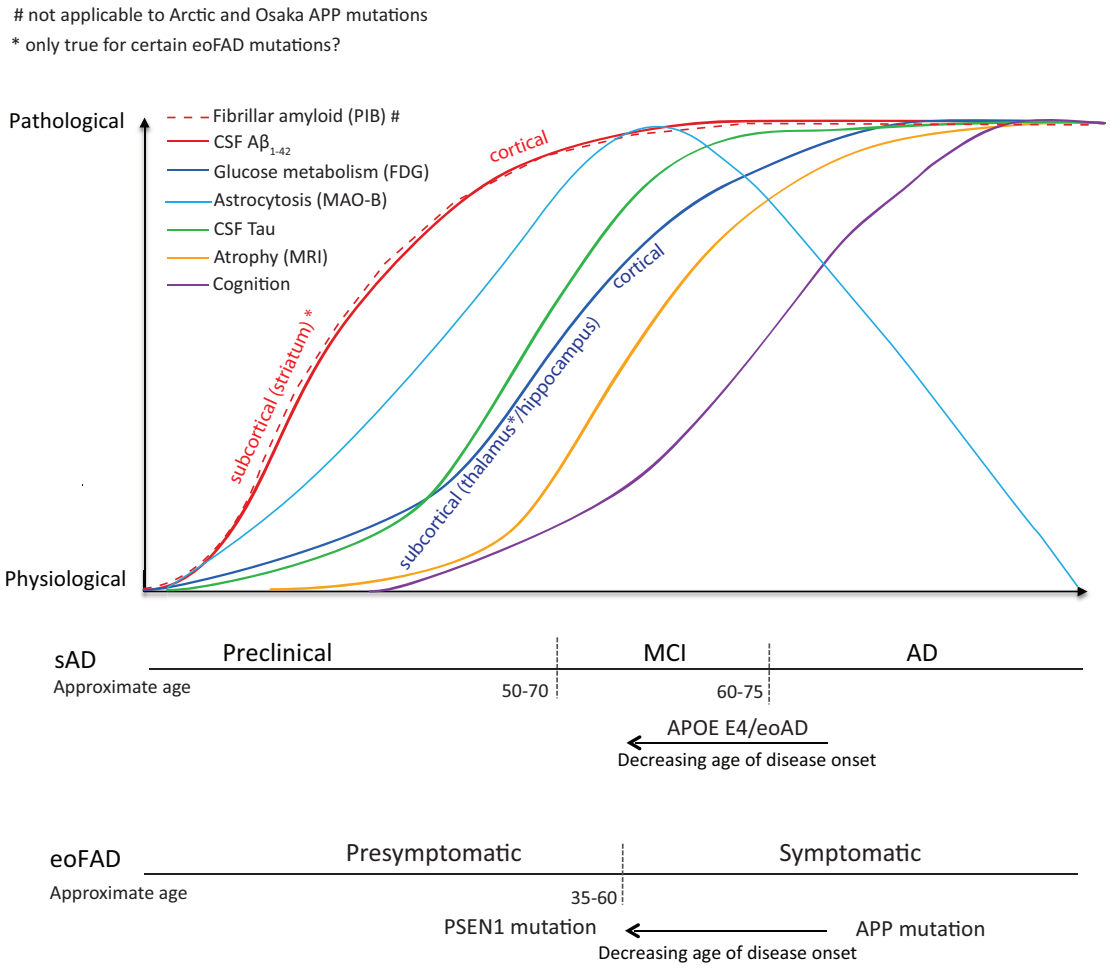
Multi-tracer PET

The author believes strongly in the potential of the multi-tracer PET approach. No other technique offers comparable possibilities of studying molecular processes in living humans and it has by that provided essential insights in disease mechanisms. The current portfolio

of available PET tracers with application in AD is impressive, yet the author is looking forward to the introduction of reliable and specific tracers for tau pathology, which would certainly contribute to the power of this collection. On the personal wish list is furthermore a study combining tracers for microglia and astrocyte activation.

Proposal for time course

As a general conclusion, based on findings in this thesis and in the context of previous studies mentioned in the Introduction, a modified hypothetical model for the time course of biomarkers of AD is proposed. The findings in this work are based on studies in groups with small numbers, sometimes even on an individual level. The presented conclusions are thus suggestions for potential facts.



Acknowledgements

I feel blessed to have met so many wonderful people during my time in Sweden and at the Karolinska Institutet. This thesis is the work of many and I am deeply grateful in particular to the following persons:

My main supervisor:
Professor Agneta Nordberg, for giving me the opportunity to work with you and your group and with truly exciting projects. For guiding me through these years as a PhD student with your enormous experience and the ability to see the tree where I see wood. Most of all, I would like to thank you though for believing in me and entrusting me with elaborate and costly studies. I have learned very much from you and I really appreciate the way you dealt with my way of working. Again and again, I am impressed by your ability to organise things in the shortest of times!

My co-supervisors:
Professor Ove Almkvist, for all the support and encouragement during the years, from patient discussions about statistics and neuropsychology in the very beginning to lessons about fish and sailing lately. When things got hot, you taught me that "it is most important not to forget to breath".
Professor Bengt Långström, for sharing with me your genuine passion for PET. Your enthusiasm is inspiring and I do hope that you will remain the most active "pensioner" I have met, the PET world would certainly loose one of its pioneers with you.

All co-authors and collaborators, especially **Henry Engler**, for being such an unorthodox researcher and person, it is great to know you. **Nenad Bogdanovic**, you were like an additional supervisor to me, always having time for a chat about brain connections and all the rest and your teaching skills are amazing. **Caroline Graff**, for being straight to the point and nice at the same time, I really appreciated our collaboration. The staff at the Uppsala PET Center, namely **Anders Wall**, who made so much of this thesis possible. You were always of great help and a pleasure to be with. **Lars "Lasse" Lindsjö** for being the knight in shining armour when things went bad. **Kerstin Heurling** for making trips to Uppsala enjoyable and all your help with modelling issues. **Johan Lilja**, **Roger** and **Jan Axelsson** for their kind help with programming and software solutions, and **Mimmi Lidholm** for her efforts with coordinating all the examinations.

Past and present members of the Division of Alzheimer Neurobiology: **Stephen Carter**, for incorporating all the Britishness I could wish for and for all your priceless help, great to have you in the office. **Ahmadul Kadir**, it feels as if we spent ages next to each other! I already miss you. **Anna Lilja and Linn Wicklund**, thank you for all the fun at and outside the lab and for being so much more than colleagues! **Amelia Marutle** for always having an open door and being so understanding, **Stina Unger-Lithner**, I will never forget how you arranged such a nice farewell dinner when I left after my exam work, you made me feel so welcome. **Taher Darreh-Shori**, also you have always an open door and time to talk. You are one of few people I know who really believe in every aspect of their research. **Jennie**, for your fine and subtle attitude. **Tamanna Mustafiz**, for always thinking of me when there are sweets, as well as **Ruiqing Ni. Yen-Bee Ng,** for being such a pleasant addition to our office line-up. **Fuxiang Bao**, for the wrestling lessons. **Anton Forsberg, Mats Nilbratt, Malahat Mousavi and Marie Svedberg**, thank you for the good times we had

together at work. Last, but definitely not least, **Agneta Lindahl**, you are the one that holds everything together in the end.

Colleagues and co-workers at NVS:
Professor Bengt Winblad, who by initiating the Swedish Brain Power Network has created a unique and encouraging research environment. I have always also appreciated that you really try to take care of KI people at conferences, it made me feel like part of a big family. **Gunilla Johansson** for taking care of all of this so well. **Professor Marianne Schultzberg**, for being such a caring person. **Professor Lars-Olof Wahlund**, you too have invited me to several really memorable activities.
All senior researchers, post-docs, current and past PhD-students, and Master students. You are way too many too list here, but you all have contributed to that I most often really enjoy being at Novum. **Eric** and **Erik**, I am more than happy that you are around, thank you for training, lunches, movies and all the good times we had together. **Anna S.** for being one of my oldest friends and such a lovely person. **PH** for all the lively discussions and dreams about a better future, **Mimmi**, **Tobbe**, **Johanna**, **Annelie** (thank you for introducing me to Norrland), **Helena**, **Maria**, **Erik S.**, **Angel, Carlos, Silvia**. **Alina Codita** for having been such an angel when I was working on this thesis. **Gabriela Spulber**, always helpful and kind. **Nodi, Behnosh, Camilla, Susanne,** you were the "old" generation when I started, now I am there. It was a great pleasure to work in the same place as you.
Und natürlich meine beiden deutschen Verbündeten, **Laura Hertwig** und **Carina Thomé**. Endlich ein paar Landsleute.

Professor Mony de Leon and **Lisa Mosconi** for the amazing time I had staying with you in NYC.

Mina vänner utanför KI: "Vardagens matadorer" **Adam, DW, Oskar, Pontus,** med flera, ni vet vilka ni är och varför ni heter matadorer. Ni har haft hand om det mesta roliga som hände under mina år i Sverige. Tusen tack för det! **Michael**, meine einzige Verbindung in die Heimat. **Gabbe**, **David** för musiken och allt omkring, **Martin, Johanna och Erika** (även om du är på KI), för er vänskap och allt vi har delat. **Samme** och **Annika** för att ni är de bästa grannar man kan ha, tack för alla kvällar hos er.

Meine Ulmer, **Uli**, **Peter** und **Daniel**. Ihr seid mir noch geblieben und ich freue mich sehr, daß wir den Kontakt halten.

Birgitta, Egil, Charlotte, Petter och Vilhelm. Tack för att ni har blivit min svenska familj. Jag är jätteglad över att jag fick er med på köpet när jag träffade Louise.

Meinen geliebten Eltern, **Hermann, Gabriele** und meinen wundervollen Brüdern **Tobias** und **Benjamin** gebührt ganz besonderer Dank. Mamma und Pappa, ohne Euch wäre nichts davon möglich gewesen. Auch wenn ich meistens weg bin, bin ich im Herzen ständig bei Euch. **Jana** und **Tanja**, ich freue mich, daß Ihr jetzt auch zur Familie gehört.

Louise, mitt hjärta! Jag ser så fram emot att äntligen få se dig mer och jag blir lycklig av tanken på vad vi kommer att uppleva tillsammans. Tack för din kärlek, ditt stöd och ditt tålamod. Jag älskar dig.

None of this research would have been possible without the generous support by the following foundations: **Swedish Medical Research Council** (project 08517), **Knut and Alice Wallenberg foundation**, **KI Stragetic Neuroscience Program, Swedish Brain Power, Swedish County Council, Swedish Alzheimer Foundation, Brain Foundation, Gun och Bertil Stohnes foundation, Old Servants foundation, Sigurd och Elsa Goljes Minne foundation, DiMi** (project EC-FP6 LSHB-200-512146), **and the Fernströms foundation**.

Lastly, but all the more, I wish to express my sincere gratitude to **all study participants and their relatives**. You have made this possible and made a crucial contribution to AD research! Thank you.

References

1. Alzheimer, A., *Über eine eigenartige Erkrankung der Hirnrinde.* Allgemeine Zeitschrift für Psychiatrie und Psychisch-gerichtliche Medizin, 1907. **64**: p. 146-8.
2. Querfurth, H.W. and F.M. LaFerla, *Alzheimer's disease.* N Engl J Med, 2010. **362**(4): p. 329-44.
3. Wimo, A., B. Winblad, and L. Jonsson, *An estimate of the total worldwide societal costs of dementia in 2005.* Alzheimers Dement, 2007. **3**(2): p. 81-91.
4. Ferri, C.P., et al., *Global prevalence of dementia: a Delphi consensus study.* Lancet, 2005. **366**(9503): p. 2112-7.
5. Brookmeyer, R., et al., *Forecasting the global burden of Alzheimer's disease.* Alzheimer's & dementia : the journal of the Alzheimer's Association, 2007. **3**(3): p. 186-91.
6. Cummings, J.L., *Alzheimer's disease.* N Engl J Med, 2004. **351**(1): p. 56-67.
7. *"sporadic".* Merriam-Webster.com, 2011. **Merriam-Webster**(Web).
8. Harvey, R.J., M. Skelton-Robinson, and M.N. Rossor, *The prevalence and causes of dementia in people under the age of 65 years.* J Neurol Neurosurg Psychiatry, 2003. **74**(9): p. 1206-9.
9. Masters, C.L., et al., *Amyloid plaque core protein in Alzheimer disease and Down syndrome.* Proc Natl Acad Sci U S A, 1985. **82**(12): p. 4245-9.
10. Glenner, G.G. and C.W. Wong, *Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein.* Biochem Biophys Res Commun, 1984. **120**(3): p. 885-90.
11. Shoji, M., et al., *Production of the Alzheimer amyloid beta protein by normal proteolytic processing.* Science, 1992. **258**(5079): p. 126-9.
12. Haass, C., et al., *Amyloid beta-peptide is produced by cultured cells during normal metabolism.* Nature, 1992. **359**(6393): p. 322-5.
13. Yoshikai, S., et al., *Genomic organization of the human amyloid beta-protein precursor gene.* Gene, 1990. **87**(2): p. 257-63.
14. Esler, W.P. and M.S. Wolfe, *A portrait of Alzheimer secretases--new features and familiar faces.* Science, 2001. **293**(5534): p. 1449-54.
15. Kojro, E. and F. Fahrenholz, *The non-amyloidogenic pathway: structure and function of alpha-secretases.* Sub-cellular biochemistry, 2005. **38**: p. 105-27.
16. Estus, S., et al., *Potentially amyloidogenic, carboxyl-terminal derivatives of the amyloid protein precursor.* Science, 1992. **255**(5045): p. 726-8.
17. Esch, F.S., et al., *Cleavage of amyloid beta peptide during constitutive processing of its precursor.* Science, 1990. **248**(4959): p. 1122-4.
18. Estus, S., T.E. Golde, and S.G. Younkin, *Normal processing of the Alzheimer's disease amyloid beta protein precursor generates potentially amyloidogenic carboxyl-terminal derivatives.* Annals of the New York Academy of Sciences, 1992. **674**: p. 138-48.
19. Golde, T.E., et al., *Processing of the amyloid protein precursor to potentially amyloidogenic derivatives.* Science, 1992. **255**(5045): p. 728-30.
20. Vassar, R., et al., *Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE.* Science, 1999. **286**(5440): p. 735-41.
21. Iwatsubo, T., et al., *Visualization of A beta 42(43) and A beta 40 in senile plaques with end-specific A beta monoclonals: evidence that an initially deposited species is A beta 42(43).* Neuron, 1994. **13**(1): p. 45-53.
22. Koffie, R.M., et al., *Oligomeric amyloid beta associates with postsynaptic densities and correlates with excitatory synapse loss near senile plaques.* Proc Natl Acad Sci U S A, 2009. **106**(10): p. 4012-7.
23. Smith, E.E. and S.M. Greenberg, *Beta-amyloid, blood vessels, and brain function.* Stroke; a journal of cerebral circulation, 2009. **40**(7): p. 2601-6.
24. Miners, J.S., et al., *Abeta-degrading enzymes in Alzheimer's disease.* Brain pathology, 2008. **18**(2): p. 240-52.
25. Mawuenyega, K.G., et al., *Decreased clearance of CNS beta-amyloid in Alzheimer's disease.* Science, 2010. **330**(6012): p. 1774.
26. Braak, H. and E. Braak, *Neuropathological stageing of Alzheimer-related changes.* Acta Neuropathol (Berl), 1991. **82**(4): p. 239-59.
27. Braak, H. and E. Braak, *Frequency of stages of Alzheimer-related lesions in different age categories.* Neurobiol Aging, 1997. **18**(4): p. 351-7.
28. Thal, D.R., et al., *Phases of A beta-deposition in the human brain and its relevance for the development of AD.* Neurology, 2002. **58**(12): p. 1791-800.

29. Thal, D.R., et al., *Sequence of Abeta-protein deposition in the human medial temporal lobe.* Journal of Neuropathology and Experimental Neurology, 2000. **59**(8): p. 733-48.
30. Terry, R.D., et al., *Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment.* Ann Neurol, 1991. **30**(4): p. 572-80.
31. Nelson, P.T., H. Braak, and W.R. Markesbery, *Neuropathology and cognitive impairment in Alzheimer disease: a complex but coherent relationship.* Journal of Neuropathology and Experimental Neurology, 2009. **68**(1): p. 1-14.
32. Giannakopoulos, P., et al., *Tangle and neuron numbers, but not amyloid load, predict cognitive status in Alzheimer's disease.* Neurology, 2003. **60**(9): p. 1495-500.
33. Arriagada, P.V., et al., *Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease.* Neurology, 1992. **42**(3 Pt 1): p. 631-9.
34. Berg, L., et al., *Clinicopathologic studies in cognitively healthy aging and Alzheimer's disease: relation of histologic markers to dementia severity, age, sex, and apolipoprotein E genotype.* Arch Neurol, 1998. **55**(3): p. 326-35.
35. Hardy, J.A. and G.A. Higgins, *Alzheimer's disease: the amyloid cascade hypothesis.* Science, 1992. **256**(5054): p. 184-5.
36. Castellani, R.J. and M.A. Smith, *Compounding artefacts with uncertainty, and an amyloid cascade hypothesis that is 'too big to fail'.* The Journal of pathology, 2011. **224**(2): p. 147-52.
37. Tanzi, R.E., *The synaptic Abeta hypothesis of Alzheimer disease.* Nature neuroscience, 2005. **8**(8): p. 977-9.
38. Hardy, J. and D.J. Selkoe, *The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics.* Science, 2002. **297**(5580): p. 353-6.
39. Lue, L.F., et al., *Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease.* The American journal of pathology, 1999. **155**(3): p. 853-62.
40. McLean, C.A., et al., *Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease.* Ann Neurol, 1999. **46**(6): p. 860-6.
41. Shankar, G.M., et al., *Natural oligomers of the Alzheimer amyloid-beta protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway.* The Journal of neuroscience : the official journal of the Society for Neuroscience, 2007. **27**(11): p. 2866-75.
42. Wang, H.W., et al., *Soluble oligomers of beta amyloid (1-42) inhibit long-term potentiation but not long-term depression in rat dentate gyrus.* Brain Res, 2002. **924**(2): p. 133-40.
43. Walsh, D.M., et al., *Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo.* Nature, 2002. **416**(6880): p. 535-9.
44. DaRocha-Souto, B., et al., *Brain oligomeric beta-amyloid but not total amyloid plaque burden correlates with neuronal loss and astrocyte inflammatory response in amyloid precursor protein/tau transgenic mice.* Journal of Neuropathology and Experimental Neurology, 2011. **70**(5): p. 360-76.
45. Lambert, M.P., et al., *Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins.* Proc Natl Acad Sci U S A, 1998. **95**(11): p. 6448-53.
46. Bao, F., et al., *Different beta-amyloid oligomer assemblies in Alzheimer brains correlate with age of disease onset and impaired cholinergic activity.* Neurobiology of aging, 2011.
47. Grundke-Iqbal, I., et al., *Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology.* Proc Natl Acad Sci U S A, 1986. **83**(13): p. 4913-7.
48. Aronov, S., et al., *Axonal tau mRNA localization coincides with tau protein in living neuronal cells and depends on axonal targeting signal.* The Journal of neuroscience : the official journal of the Society for Neuroscience, 2001. **21**(17): p. 6577-87.
49. Konzack, S., et al., *Swimming against the tide: mobility of the microtubule-associated protein tau in neurons.* The Journal of neuroscience : the official journal of the Society for Neuroscience, 2007. **27**(37): p. 9916-27.
50. Ittner, L.M., et al., *Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models.* Cell, 2010. **142**(3): p. 387-97.
51. Gotz, J., L.M. Ittner, and S. Kins, *Do axonal defects in tau and amyloid precursor protein transgenic animals model axonopathy in Alzheimer's disease?* J Neurochem, 2006. **98**(4): p. 993-1006.
52. Ittner, L.M. and J. Gotz, *Amyloid-beta and tau--a toxic pas de deux in Alzheimer's disease.* Nature reviews. Neuroscience, 2011. **12**(2): p. 65-72.
53. Gotz, J., et al., *Somatodendritic localization and hyperphosphorylation of tau protein in transgenic mice expressing the longest human brain tau isoform.* The EMBO journal, 1995. **14**(7): p. 1304-13.

54. Iqbal, K., et al., *Tau pathology in Alzheimer disease and other tauopathies.* Biochimica et biophysica acta, 2005. **1739**(2-3): p. 198-210.
55. Braak, H., et al., *Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry.* Acta Neuropathol (Berl), 2006. **112**(4): p. 389-404.
56. Alafuzoff, I., et al., *Staging of neurofibrillary pathology in Alzheimer's disease: a study of the BrainNet Europe Consortium.* Brain pathology, 2008. **18**(4): p. 484-96.
57. Schonheit, B., R. Zarski, and T.G. Ohm, *Spatial and temporal relationships between plaques and tangles in Alzheimer-pathology.* Neurobiol Aging, 2004. **25**(6): p. 697-711.
58. Giannakopoulos, P., et al., *Assessing the cognitive impact of Alzheimer disease pathology and vascular burden in the aging brain: the Geneva experience.* Acta Neuropathol (Berl), 2007. **113**(1): p. 1-12.
59. Braak, H. and K. Del Tredici, *The pathological process underlying Alzheimer's disease in individuals under thirty.* Acta Neuropathol (Berl), 2011. **121**(2): p. 171-81.
60. Braak, H. and K. Del Tredici, *Alzheimer's pathogenesis: is there neuron-to-neuron propagation?* Acta Neuropathol (Berl), 2011. **121**(5): p. 589-95.
61. Griffin, W.S., et al., *Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease.* Proc Natl Acad Sci U S A, 1989. **86**(19): p. 7611-5.
62. McGeer, P.L., et al., *Anti-inflammatory drugs and Alzheimer disease.* Lancet, 1990. **335**(8696): p. 1037.
63. McGeer, P.L., M. Schulzer, and E.G. McGeer, *Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies.* Neurology, 1996. **47**(2): p. 425-32.
64. McGeer, E.G. and P.L. McGeer, *Neuroinflammation in Alzheimer's disease and mild cognitive impairment: a field in its infancy.* Journal of Alzheimer's disease : JAD, 2010. **19**(1): p. 355-61.
65. Nimmerjahn, A., F. Kirchhoff, and F. Helmchen, *Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo.* Science, 2005. **308**(5726): p. 1314-8.
66. Kreutzberg, G.W., *Microglia: a sensor for pathological events in the CNS.* Trends Neurosci, 1996. **19**(8): p. 312-8.
67. Heneka, M.T. and M.K. O'Banion, *Inflammatory processes in Alzheimer's disease.* Journal of neuroimmunology, 2007. **184**(1-2): p. 69-91.
68. Mrak, R.E., *Neuropathology and the neuroinflammation idea.* Journal of Alzheimer's disease : JAD, 2009. **18**(3): p. 473-81.
69. Griffin, W.S., et al., *Interleukin-1 mediates Alzheimer and Lewy body pathologies.* Journal of neuroinflammation, 2006. **3**: p. 5.
70. Del Bo, R., et al., *Reciprocal control of inflammatory cytokines, IL-1 and IL-6, and beta-amyloid production in cultures.* Neurosci Lett, 1995. **188**(1): p. 70-4.
71. Halassa, M.M. and P.G. Haydon, *Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior.* Annual review of physiology, 2010. **72**: p. 335-55.
72. Barros, L.F., O.H. Porras, and C.X. Bittner, *Why glucose transport in the brain matters for PET.* Trends Neurosci, 2005. **28**(3): p. 117-9.
73. Magistretti, P.J., *Neuron-glia metabolic coupling and plasticity.* The Journal of experimental biology, 2006. **209**(Pt 12): p. 2304-11.
74. Suzuki, A., et al., *Astrocyte-neuron lactate transport is required for long-term memory formation.* Cell, 2011. **144**(5): p. 810-23.
75. Allaman, I., et al., *Amyloid-beta aggregates cause alterations of astrocytic metabolic phenotype: impact on neuronal viability.* The Journal of neuroscience : the official journal of the Society for Neuroscience, 2010. **30**(9): p. 3326-38.
76. Saura, J., et al., *Increased monoamine oxidase B activity in plaque-associated astrocytes of Alzheimer brains revealed by quantitative enzyme radioautography.* Neuroscience, 1994. **62**(1): p. 15-30.
77. Yu, W.F., et al., *High selective expression of alpha7 nicotinic receptors on astrocytes in the brains of patients with sporadic Alzheimer's disease and patients carrying Swedish APP 670/671 mutation: a possible association with neuritic plaques.* Exp Neurol, 2005. **192**(1): p. 215-25.
78. Cairns, N.J., et al., *Astrocytosis, beta A4-protein deposition and paired helical filament formation in Alzheimer's disease.* Journal of the neurological sciences, 1992. **112**(1-2): p. 68-75.
79. Yamaguchi, H., et al., *Diffuse plaques associated with astroglial amyloid beta protein, possibly showing a disappearing stage of senile plaques.* Acta Neuropathol (Berl), 1998. **95**(3): p. 217-22.
80. Shaffer, L.M., et al., *Amyloid beta protein (A beta) removal by neuroglial cells in culture.* Neurobiol Aging, 1995. **16**(5): p. 737-45.

81. Lasagna-Reeves, C.A. and R. Kayed, *Astrocytes contain amyloid-beta annular protofibrils in Alzheimer's disease brains.* FEBS letters, 2011.
82. Nagele, R.G., et al., *Astrocytes accumulate A beta 42 and give rise to astrocytic amyloid plaques in Alzheimer disease brains.* Brain Res, 2003. **971**(2): p. 197-209.
83. Wyss-Coray, T., et al., *Adult mouse astrocytes degrade amyloid-beta in vitro and in situ.* Nat Med, 2003. **9**(4): p. 453-7.
84. Rodriguez, J.J., et al., *Astroglia in dementia and Alzheimer's disease.* Cell death and differentiation, 2009. **16**(3): p. 378-85.
85. Rossner, S., et al., *Alzheimer's disease beta-secretase BACE1 is not a neuron-specific enzyme.* J Neurochem, 2005. **92**(2): p. 226-34.
86. Lowenberg, K. and R. Waggoner, *Familial organic psychosis (Alzheimer's type).* Arch Neurol Psychiatr, 1934. **31**: p. 737-754.
87. Rosen, W.G., R.C. Mohs, and K.L. Davis, *A new rating scale for Alzheimer's disease.* Am J Psychiatry, 1984. **141**(11): p. 1356-64.
88. Goate, A., et al., *Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease.* Nature, 1991. **349**(6311): p. 704-706.
89. Sherrington, R., et al., *Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease.* Nature, 1995. **375**(6534): p. 754-760.
90. Levy-Lahad, E., et al., *Candidate gene for the chromosome 1 familial Alzheimer's disease locus.* Science, 1995. **269**(5226): p. 973-977.
91. Rogaev, E.I., et al., *Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene.* Nature, 1995. **376**(6543): p. 775-778.
92. Wisniewski, K.E., H.M. Wisniewski, and G.Y. Wen, *Occurrence of neuropathological changes and dementia of Alzheimer's disease in Down's syndrome.* Ann Neurol, 1985. **17**(3): p. 278-82.
93. Raux, G., et al., *Molecular diagnosis of autosomal dominant early onset Alzheimer's disease: an update.* Journal of medical genetics, 2005. **42**(10): p. 793-5.
94. Di Fede, G., et al., *A recessive mutation in the APP gene with dominant-negative effect on amyloidogenesis.* Science, 2009. **323**(5920): p. 1473-7.
95. Tomiyama, T., et al., *A new amyloid beta variant favoring oligomerization in Alzheimer's-type dementia.* Ann Neurol, 2008. **63**(3): p. 377-87.
96. Brouwers, N., K. Sleegers, and C. Van Broeckhoven, *Molecular genetics of Alzheimer's disease: an update.* Annals of medicine, 2008. **40**(8): p. 562-83.
97. Nilsberth, C., et al., *The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Abeta protofibril formation.* Nat Neurosci, 2001. **4**(9): p. 887-93.
98. Basun, H., et al., *Clinical and neuropathological features of the arctic APP gene mutation causing early-onset Alzheimer disease.* Arch Neurol, 2008. **65**(4): p. 499-505.
99. Ryan, N.S. and M.N. Rossor, *Correlating familial Alzheimer's disease gene mutations with clinical phenotype.* Biomarkers in medicine, 2010. **4**(1): p. 99-112.
100. Ashford, J.W., *APOE genotype effects on Alzheimer's disease onset and epidemiology.* Journal of molecular neuroscience : MN, 2004. **23**(3): p. 157-65.
101. Daw, E.W., et al., *The number of trait loci in late-onset Alzheimer disease.* American journal of human genetics, 2000. **66**(1): p. 196-204.
102. Rogaeva, E., et al., *The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease.* Nature Genetics, 2007. **39**(2): p. 168-77.
103. *Diagnostic and statistical manual of mental disorders, 4th ed.* DSM-IV, 1994.
104. McKhann, G., et al., *Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease.* Neurology, 1984. **34**(7): p. 939-44.
105. Chui, H. and L. A-E., *Clinical criteria for dementia subtypes. In: Qizilbash N, Schneider L, Brodaty H, et al. eds. Evidence-based dementia practice.* Oxford, England: Blackwell Science, 2002: p. 106-19.
106. Ballard, C. and B. C., *Criteria in the diagnosis of dementia. In: Burns A, O'Brien J, Ames D eds.* Dementia, 3rd edn. London: Hodder, 2005: p. 24-37.
107. Folstein, M.F., S.E. Folstein, and P.R. McHugh, *"Mini-mental state". A practical method for grading the cognitive state of patients for the clinician.* J Psychiatr Res, 1975. **12**(3): p. 189-98.
108. Hughes, C.P., et al., *A new clinical scale for the staging of dementia.* Br J Psychiatry, 1982. **140**: p. 566-72.
109. Almkvist, O. and L. Backman, *Detection and staging of early clinical dementia.* Acta Neurol Scand, 1993. **88**(1): p. 10-5.

110. Welsh, K.A., et al., *Detection and staging of dementia in Alzheimer's disease. Use of the neuropsychological measures developed for the Consortium to Establish a Registry for Alzheimer's Disease.* Arch Neurol, 1992. **49**(5): p. 448-52.
111. Berg, L., *Clinical Dementia Rating (CDR).* Psychopharmacol Bull, 1988. **24**(4): p. 637-9.
112. Weyer, G., et al., *Alzheimer's Disease Assessment Scale: reliability and validity in a multicenter clinical trial.* Int Psychogeriatr, 1997. **9**(2): p. 123-38.
113. Ihl, R., et al., *Detecting treatment effects with combinations of the ADAS-cog items in patients with mild and moderate Alzheimer's disease.* International journal of geriatric psychiatry, 2011.
114. Salmon, D.P. and M.W. Bondi, *Neuropsychological assessment of dementia.* Annu Rev Psychol, 2009. **60**: p. 257-82.
115. Almkvist, O., G. Brane, and A. Johanson, *Neuropsychological assessment of dementia: state of the art.* Acta Neurol Scand Suppl, 1996. **168**: p. 45-9.
116. Salmon, D.P., *Disorders of memroy in Alzheimer's disease. In: Cermak LS, editor.* Handbook of Neuropsychology, 2000. **Vol 2: Memory and Its Disorders**(2nd Amsterdam: Elsevier): p. 155-95.
117. Race, E., M.M. Keane, and M. Verfaellie, *Medial temporal lobe damage causes deficits in episodic memory and episodic future thinking not attributable to deficits in narrative construction.* The Journal of neuroscience : the official journal of the Society for Neuroscience, 2011. **31**(28): p. 10262-9.
118. Weintraub, S., et al., *The Alzheimer's Disease Centers' Uniform Data Set (UDS): the neuropsychologic test battery.* Alzheimer Dis Assoc Disord, 2009. **23**(2): p. 91-101.
119. Wechsler, D., *Wechsler Adult Intelligence Scale - Revised*, 1981, Psychological Corporation: New York.
120. Lezak, M., D. Howieson, and L. DW., in *Neuropsychological Assessment (Fifth Edition)*2004, Oxford University Press: New York.
121. Kaplan, E., H. Goodglass, and S. Weintraub, *The Boston Naming Test*, 1983, Lea and Febiger: Philadelphia.
122. Petersen, R.C., et al., *Mild cognitive impairment: clinical characterization and outcome.* Arch Neurol, 1999. **56**(3): p. 303-8.
123. Petersen, R.C., *Mild cognitive impairment as a diagnostic entity.* J Intern Med, 2004. **256**(3): p. 183-94.
124. Winblad, B., et al., *Mild cognitive impairment--beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment.* J Intern Med, 2004. **256**(3): p. 240-6.
125. Petersen, R.C., et al., *Mild cognitive impairment: ten years later.* Arch Neurol, 2009. **66**(12): p. 1447-55.
126. Fischer, P., et al., *Conversion from subtypes of mild cognitive impairment to Alzheimer dementia.* Neurology, 2007. **68**(4): p. 288-91.
127. Solfrizzi, V., et al., *Vascular risk factors, incidence of MCI, and rates of progression to dementia.* Neurology, 2004. **63**(10): p. 1882-91.
128. Busse, A., et al., *Mild cognitive impairment: long-term course of four clinical subtypes.* Neurology, 2006. **67**(12): p. 2176-85.
129. AJ., A., et al., *Biomarkers and surrogate endpoints: preferred definitions and conceptual framework.* Clinical pharmacology and therapeutics, 2001. **69**(3): p. 89-95.
130. Whitwell, J.L., et al., *MRI correlates of neurofibrillary tangle pathology at autopsy: a voxel-based morphometry study.* Neurology, 2008. **71**(10): p. 743-9.
131. Mueller, S.G., N. Schuff, and M.W. Weiner, *Evaluation of treatment effects in Alzheimer's and other neurodegenerative diseases by MRI and MRS.* NMR in biomedicine, 2006. **19**(6): p. 655-68.
132. Jack, C.R., Jr., et al., *Medial temporal atrophy on MRI in normal aging and very mild Alzheimer's disease.* Neurology, 1997. **49**(3): p. 786-94.
133. Laakso, M.P., et al., *MRI of the hippocampus in Alzheimer's disease: sensitivity, specificity, and analysis of the incorrectly classified subjects.* Neurobiol Aging, 1998. **19**(1): p. 23-31.
134. Xu, Y., et al., *Usefulness of MRI measures of entorhinal cortex versus hippocampus in AD.* Neurology, 2000. **54**(9): p. 1760-7.
135. DeCarli, C., et al., *Qualitative estimates of medial temporal atrophy as a predictor of progression from mild cognitive impairment to dementia.* Arch Neurol, 2007. **64**(1): p. 108-15.
136. Barnes, J., et al., *A meta-analysis of hippocampal atrophy rates in Alzheimer's disease.* Neurobiol Aging, 2009. **30**(11): p. 1711-23.

137. Mosconi, L., et al., *Hypometabolism exceeds atrophy in presymptomatic early-onset familial Alzheimer's disease.* J Nucl Med, 2006. **47**(11): p. 1778-86.
138. Wahlund, L.O., et al., *A follow-up study of the family with the Swedish APP 670/671 Alzheimer's disease mutation.* Dement Geriatr Cogn Disord, 1999. **10**(6): p. 526-33.
139. Fortea, J., et al., *Increased cortical thickness and caudate volume precede atrophy in PSEN1 mutation carriers.* Journal of Alzheimer's disease : JAD, 2010. **22**(3): p. 909-22.
140. Sheline, Y.I., et al., *Amyloid plaques disrupt resting state default mode network connectivity in cognitively normal elderly.* Biol Psychiatry, 2010. **67**(6): p. 584-7.
141. Oh, H., et al., *beta-Amyloid affects frontal and posterior brain networks in normal aging.* Neuroimage, 2011. **54**(3): p. 1887-95.
142. Brownell, G. and W. Sweet, *Localization of brain tumors with positron emitters.* Nucleonics, 1953. **11**: p. 40-45.
143. *"tomography".* Merriam-Webster.com, 2011. **Merriam-Webster**(Web).
144. Kadir, A., et al., *Effect of phenserine treatment on brain functional activity and amyloid in Alzheimer's disease.* Ann Neurol, 2008. **63**(5): p. 621-31.
145. Kadir, A., et al., *PET imaging of the in vivo brain acetylcholinesterase activity and nicotine binding in galantamine-treated patients with AD.* Neurobiol Aging, 2008. **29**(8): p. 1204-17.
146. Kadir, A., et al., *Changes in brain 11C-nicotine binding sites in patients with mild Alzheimer's disease following rivastigmine treatment as assessed by PET.* Psychopharmacology, 2007. **191**(4): p. 1005-14.
147. Keller, C., et al., *Long-term effects of galantamine treatment on brain functional activities as measured by PET in Alzheimer's disease patients.* Journal of Alzheimer's disease : JAD, 2011. **24**(1): p. 109-23.
148. Paans, A., et al., *Positron emission tomography: the conceptual idea using a multidisciplinary approach.* Methods, 2002. **27**: p. 195-207.
149. Lewellen, T.K., *Recent developments in PET detector technology.* Physics in medicine and biology, 2008. **53**(17): p. R287-317.
150. Surti, S., et al., *Performance of Philips Gemini TF PET/CT scanner with special consideration for its time-of-flight imaging capabilities.* Journal of nuclear medicine : official publication, Society of Nuclear Medicine, 2007. **48**(3): p. 471-80.
151. Thie, J.A., *Understanding the standardized uptake value, its methods, and implications for usage.* Journal of nuclear medicine : official publication, Society of Nuclear Medicine, 2004. **45**(9): p. 1431-4.
152. Herholz, K., P. Herscovitch, and W.D. Heiss, *NeuroPET*2004, Berlin: Springer-Verlag.
153. Wahab, P.J., et al., *Venous versus arterialised venous blood for assessment of blood glucose levels during glucose clamping: comparison in healthy men.* Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme, 1992. **24**(12): p. 576-9.
154. Fischl, B. and A.M. Dale, *Measuring the thickness of the human cerebral cortex from magnetic resonance images.* Proc Natl Acad Sci U S A, 2000. **97**(20): p. 11050-5.
155. Rousset, O.G., Y. Ma, and A.C. Evans, *Correction for partial volume effects in PET: principle and validation.* Journal of nuclear medicine : official publication, Society of Nuclear Medicine, 1998. **39**(5): p. 904-11.
156. Meltzer, C.C., et al., *Regional hypometabolism in Alzheimer's disease as measured by positron emission tomography after correction for effects of partial volume averaging.* Neurology, 1996. **47**(2): p. 454-61.
157. Hammers, A., et al., *Statistical neuroanatomy of the human inferior frontal gyrus and probabilistic atlas in a standard stereotaxic space.* Hum Brain Mapp, 2007. **28**(1): p. 34-48.
158. Frackowiak, R.S., et al., *Regional cerebral oxygen supply and utilization in dementia. A clinical and physiological study with oxygen-15 and positron tomography.* Brain : a journal of neurology, 1981. **104**(Pt 4): p. 753-78.
159. de Leon, M.J., et al., *Positron emission tomographic studies of aging and Alzheimer disease.* AJNR Am J Neuroradiol, 1983. **4**(3): p. 568-71.
160. Foster, N.L., et al., *Alzheimer's disease: focal cortical changes shown by positron emission tomography.* Neurology, 1983. **33**(8): p. 961-5.
161. Friedland, R.P., et al., *Regional cerebral metabolic alterations in dementia of the Alzheimer type: positron emission tomography with [18F]fluorodeoxyglucose.* J Comput Assist Tomogr, 1983. **7**(4): p. 590-8.
162. Kaneko, K., et al., *Posterior cingulate hypoperfusion in Alzheimer's disease, senile dementia of Alzheimer type, and other dementias evaluated by three-dimensional stereotactic surface projections using Tc-99m HMPAO SPECT.* Clinical nuclear medicine, 2004. **29**(6): p. 362-6.

163. Tang, B.N., et al., *Diagnosis of suspected Alzheimer's disease is improved by automated analysis of regional cerebral blood flow.* Eur J Nucl Med Mol Imaging, 2004. **31**(11): p. 1487-94.
164. Sojkova, J., et al., *Longitudinal cerebral blood flow and amyloid deposition: an emerging pattern?* Journal of nuclear medicine : official publication, Society of Nuclear Medicine, 2008. **49**(9): p. 1465-71.
165. Ishii, K. and S. Minoshima, *PET is better than perfusion SPECT for early diagnosis of Alzheimer's disease -- for.* Eur J Nucl Med Mol Imaging, 2005. **32**(12): p. 1463-5.
166. Blomquist, G., et al., *Unidirectional Influx and Net Accumulation of PIB.* The open neuroimaging journal, 2008. **2**: p. 114-25.
167. Meyer, P.T., et al., *Dual-biomarker imaging of regional cerebral amyloid load and neuronal activity in dementia with PET and 11C-labeled Pittsburgh compound B.* Journal of nuclear medicine : official publication, Society of Nuclear Medicine, 2011. **52**(3): p. 393-400.
168. Rostomian, A.H., et al., *Early 11C-PIB frames and 18F-FDG PET measures are comparable: a study validated in a cohort of AD and FTLD patients.* Journal of nuclear medicine : official publication, Society of Nuclear Medicine, 2011. **52**(2): p. 173-9.
169. Vannucci, S.J., F. Maher, and I.A. Simpson, *Glucose transporter proteins in brain: delivery of glucose to neurons and glia.* Glia, 1997. **21**(1): p. 2-21.
170. Herholz, K., *PET studies in dementia.* Annals of nuclear medicine, 2003. **17**(2): p. 79-89.
171. Mosconi, L., *Brain glucose metabolism in the early and specific diagnosis of Alzheimer's disease. FDG-PET studies in MCI and AD.* Eur J Nucl Med Mol Imaging, 2005. **32**(4): p. 486-510.
172. Minoshima, S., et al., *Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease.* Ann Neurol, 1997. **42**(1): p. 85-94.
173. Kim, E.J., et al., *Glucose metabolism in early onset versus late onset Alzheimer's disease: an SPM analysis of 120 patients.* Brain : a journal of neurology, 2005. **128**(Pt 8): p. 1790-801.
174. Sakamoto, S., et al., *Differences in cerebral metabolic impairment between early and late onset types of Alzheimer's disease.* Journal of the neurological sciences, 2002. **200**(1-2): p. 27-32.
175. Jagust, W., et al., *What does fluorodeoxyglucose PET imaging add to a clinical diagnosis of dementia?* Neurology, 2007. **69**(9): p. 871-7.
176. Silverman, D.H., et al., *Positron emission tomography in evaluation of dementia: Regional brain metabolism and long-term outcome.* JAMA : the journal of the American Medical Association, 2001. **286**(17): p. 2120-7.
177. Herholz, K., et al., *Impairment of neocortical metabolism predicts progression in Alzheimer's disease.* Dement Geriatr Cogn Disord, 1999. **10**(6): p. 494-504.
178. Desgranges, B., et al., *The neural substrates of episodic memory impairment in Alzheimer's disease as revealed by FDG-PET: relationship to degree of deterioration.* Brain : a journal of neurology, 2002. **125**(Pt 5): p. 1116-24.
179. Landau, S.M., et al., *Associations between cognitive, functional, and FDG-PET measures of decline in AD and MCI.* Neurobiol Aging, 2011. **32**(7): p. 1207-18.
180. Arnaiz, E., et al., *Impaired cerebral glucose metabolism and cognitive functioning predict deterioration in mild cognitive impairment.* Neuroreport, 2001. **12**(4): p. 851-5.
181. Chetelat, G., et al., *Mild cognitive impairment: Can FDG-PET predict who is to rapidly convert to Alzheimer's disease?* Neurology, 2003. **60**(8): p. 1374-7.
182. de Leon, M.J., et al., *Prediction of cognitive decline in normal elderly subjects with 2-[(18)F]fluoro-2-deoxy-D-glucose/poitron-emission tomography (FDG/PET).* Proc Natl Acad Sci U S A, 2001. **98**(19): p. 10966-71.
183. Drzezga, A., et al., *Cerebral metabolic changes accompanying conversion of mild cognitive impairment into Alzheimer's disease: a PET follow-up study.* Eur J Nucl Med Mol Imaging, 2003. **30**(8): p. 1104-13.
184. Nestor, P.J., et al., *Limbic hypometabolism in Alzheimer's disease and mild cognitive impairment.* Ann Neurol, 2003. **54**(3): p. 343-51.
185. Mosconi, L., et al., *FDG-PET changes in brain glucose metabolism from normal cognition to pathologically verified Alzheimer's disease.* Eur J Nucl Med Mol Imaging, 2009. **36**(5): p. 811-22.
186. Jagust, W., et al., *Brain imaging evidence of preclinical Alzheimer's disease in normal aging.* Ann Neurol, 2006. **59**(4): p. 673-81.
187. Small, G.W., et al., *Apolipoprotein E type 4 allele and cerebral glucose metabolism in relatives at risk for familial Alzheimer disease.* JAMA : the journal of the American Medical Association, 1995. **273**(12): p. 942-7.
188. Reiman, E.M., et al., *Preclinical evidence of Alzheimer's disease in persons homozygous for the epsilon 4 allele for apolipoprotein E.* N Engl J Med, 1996. **334**(12): p. 752-8.

189. Small, G.W., et al., *Cerebral metabolic and cognitive decline in persons at genetic risk for Alzheimer's disease.* Proc Natl Acad Sci U S A, 2000. **97**(11): p. 6037-42.
190. Mosconi, L., et al., *Hypometabolism and altered cerebrospinal fluid markers in normal apolipoprotein E E4 carriers with subjective memory complaints.* Biol Psychiatry, 2008. **63**(6): p. 609-18.
191. Kennedy, A.M., et al., *Chromosome 14 linked familial Alzheimer's disease. A clinico-pathological study of a single pedigree.* Brain : a journal of neurology, 1995. **118 (Pt 1)**: p. 185-205.
192. Kennedy, A.M., et al., *Deficits in cerebral glucose metabolism demonstrated by positron emission tomography in individuals at risk of familial Alzheimer's disease.* Neurosci Lett, 1995. **186**(1): p. 17-20.
193. Ringman, J.M., et al., *Biochemical, neuropathological, and neuroimaging characteristics of early-onset Alzheimer's disease due to a novel PSEN1 mutation.* Neurosci Lett, 2011. **487**(3): p. 287-92.
194. Perani, D., et al., *PET study in subjects from two Italian FAD families with APP717 Val to Ileu mutation.* Eur J Neurosci, 1997. **4**: p. 214-220.
195. Mosconi, L., et al., *Maternal family history of Alzheimer's disease predisposes to reduced brain glucose metabolism.* Proc Natl Acad Sci U S A, 2007. **104**(48): p. 19067-72.
196. Mosconi, L., et al., *Declining brain glucose metabolism in normal individuals with a maternal history of Alzheimer disease.* Neurology, 2009. **72**(6): p. 513-20.
197. Valla, J., J.D. Berndt, and F. Gonzalez-Lima, *Energy hypometabolism in posterior cingulate cortex of Alzheimer's patients: superficial laminar cytochrome oxidase associated with disease duration.* The Journal of neuroscience : the official journal of the Society for Neuroscience, 2001. **21**(13): p. 4923-30.
198. Valla, J., et al., *Reduced posterior cingulate mitochondrial activity in expired young adult carriers of the APOE epsilon4 allele, the major late-onset Alzheimer's susceptibility gene.* Journal of Alzheimer's disease : JAD, 2010. **22**(1): p. 307-13.
199. Alexander, G.E., et al., *Longitudinal PET Evaluation of Cerebral Metabolic Decline in Dementia: A Potential Outcome Measure in Alzheimer's Disease Treatment Studies.* Am J Psychiatry, 2002. **159**(5): p. 738-45.
200. Braak, H., et al., *Neuropathological hallmarks of Alzheimer's and Parkinson's diseases.* Prog Brain Res, 1998. **117**: p. 267-85.
201. Rinne, J.O., et al., *Brain acetylcholinesterase activity in mild cognitive impairment and early Alzheimer's disease.* J Neurol Neurosurg Psychiatry, 2003. **74**(1): p. 113-5.
202. Rinne, J.O. and K. Nagren, *Positron emission tomography in at risk patients and in the progression of mild cognitive impairment to Alzheimer's disease.* Journal of Alzheimer's disease : JAD, 2010. **19**(1): p. 291-300.
203. Madsen, K., et al., *Cognitive function is related to fronto-striatal serotonin transporter levels--a brain PET study in young healthy subjects.* Psychopharmacology, 2011. **213**(2-3): p. 573-81.
204. Marner, L., et al., *The reduction of baseline serotonin 2A receptors in mild cognitive impairment is stable at two-year follow-up.* Journal of Alzheimer's disease : JAD, 2011. **23**(3): p. 453-9.
205. Mathis, C.A., et al., *A lipophilic thioflavin-T derivative for positron emission tomography (PET) imaging of amyloid in brain.* Bioorg Med Chem Lett, 2002. **12**(3): p. 295-8.
206. Bacskai, B.J., et al., *Four-dimensional multiphoton imaging of brain entry, amyloid binding, and clearance of an amyloid-beta ligand in transgenic mice.* Proc Natl Acad Sci U S A, 2003. **100**(21): p. 12462-7.
207. Ikonomovic, M.D., et al., *Post-mortem correlates of in vivo PiB-PET amyloid imaging in a typical case of Alzheimer's disease.* Brain, 2008. **131**(Pt 6): p. 1630-45.
208. Lockhart, A., et al., *PIB is a non-specific imaging marker of amyloid-beta (Abeta) peptide-related cerebral amyloidosis.* Brain : a journal of neurology, 2007. **130**(Pt 10): p. 2607-15.
209. Kadir, A., et al., *Positron emission tomography imaging and clinical progression in relation to molecular pathology in the first Pittsburgh Compound B positron emission tomography patient with Alzheimer's disease.* Brain, 2011. **134**(Pt 1): p. 301-17.
210. Svedberg, M.M., et al., *[(11)C]PIB-amyloid binding and levels of Abeta40 and Abeta42 in postmortem brain tissue from Alzheimer patients.* Neurochemistry international, 2009. **54**(5-6): p. 347-57.
211. Klunk, W.E., et al., *Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B.* Ann Neurol, 2004. **55**(3): p. 306-19.
212. Archer, H.A., et al., *Amyloid load and cerebral atrophy in Alzheimer's disease: an 11C-PIB positron emission tomography study.* Ann Neurol, 2006. **60**(1): p. 145-7.

213. Kemppainen, N.M., et al., *Voxel-based analysis of PET amyloid ligand [11C]PIB uptake in Alzheimer disease.* Neurology, 2006. **67**(9): p. 1575-80.
214. Rowe, C.C., et al., *Imaging beta-amyloid burden in aging and dementia.* Neurology, 2007. **68**(20): p. 1718-25.
215. Engler, H., et al., *Two-year follow-up of amyloid deposition in patients with Alzheimer's disease.* Brain, 2006. **129**(Pt 11): p. 2856-66.
216. Kadir, A., et al., *Dynamic changes in PET amyloid and FDG imaging at different stages of Alzheimer's disease.* Neurobiol Aging, 2010.
217. Rabinovici, G.D., et al., *Increased metabolic vulnerability in early-onset Alzheimer's disease is not related to amyloid burden.* Brain : a journal of neurology, 2010. **133**(Pt 2): p. 512-28.
218. Furst, A.J., et al., *Cognition, glucose metabolism and amyloid burden in Alzheimer's disease.* Neurobiology of aging, 2010.
219. Forsberg, A., et al., *High PIB retention in Alzheimer's disease is an early event with complex relationship with CSF biomarkers and functional parameters.* Curr Alzheimer Res, 2010. **7**(1): p. 56-66.
220. Forsberg, A., et al., *PET imaging of amyloid deposition in patients with mild cognitive impairment.* Neurobiol Aging, 2008. **29**(10): p. 1456-65.
221. Fagan, A.M., et al., *Inverse relation between in vivo amyloid imaging load and cerebrospinal fluid Abeta42 in humans.* Ann Neurol, 2006. **59**(3): p. 512-9.
222. Cairns, N.J., et al., *Absence of Pittsburgh compound B detection of cerebral amyloid beta in a patient with clinical, cognitive, and cerebrospinal fluid markers of Alzheimer disease: a case report.* Archives of neurology, 2009. **66**(12): p. 1557-62.
223. Clark, C.M., et al., *Cerebrospinal fluid tau and beta-amyloid: how well do these biomarkers reflect autopsy-confirmed dementia diagnoses?* Arch Neurol, 2003. **60**(12): p. 1696-702.
224. Reiman, E.M., et al., *Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease.* Proc Natl Acad Sci U S A, 2009. **106**(16): p. 6820-5.
225. Drzezga, A., et al., *Effect of APOE genotype on amyloid plaque load and gray matter volume in Alzheimer disease.* Neurology, 2009. **72**(17): p. 1487-94.
226. Klunk, W.E., et al., *Amyloid deposition begins in the striatum of presenilin-1 mutation carriers from two unrelated pedigrees.* J Neurosci, 2007. **27**(23): p. 6174-84.
227. Villemagne, V.L., et al., *High striatal amyloid beta-peptide deposition across different autosomal Alzheimer disease mutation types.* Arch Neurol, 2009. **66**(12): p. 1537-44.
228. Koivunen, J., et al., *PET amyloid ligand [11C]PIB uptake shows predominantly striatal increase in variant Alzheimer's disease.* Brain, 2008. **131**(Pt 7): p. 1845-53.
229. Shoghi-Jadid, K., et al., *Localization of neurofibrillary tangles and beta-amyloid plaques in the brains of living patients with Alzheimer disease.* Am J Geriatr Psychiatry, 2002. **10**(1): p. 24-35.
230. Tolboom, N., et al., *Detection of Alzheimer pathology in vivo using both 11C-PIB and 18F-FDDNP PET.* Journal of nuclear medicine : official publication, Society of Nuclear Medicine, 2009. **50**(2): p. 191-7.
231. Rowe, C.C., et al., *Imaging of amyloid beta in Alzheimer's disease with 18F-BAY94-9172, a novel PET tracer: proof of mechanism.* Lancet neurology, 2008. **7**(2): p. 129-35.
232. Vandenberghe, R., et al., *18F-flutemetamol amyloid imaging in Alzheimer disease and mild cognitive impairment: a phase 2 trial.* Ann Neurol, 2010. **68**(3): p. 319-29.
233. Clark, C.M., et al., *Use of florbetapir-PET for imaging beta-amyloid pathology.* JAMA : the journal of the American Medical Association, 2011. **305**(3): p. 275-83.
234. Banati, R.B., *Visualising microglial activation in vivo.* Glia, 2002. **40**(2): p. 206-17.
235. Lang, S., *The role of peripheral benzodiazepine receptors (PBRs) in CNS pathophysiology.* Current medicinal chemistry, 2002. **9**(15): p. 1411-5.
236. Groom, G.N., et al., *PET of peripheral benzodiazepine binding sites in the microgliosis of Alzheimer's disease.* Journal of nuclear medicine : official publication, Society of Nuclear Medicine, 1995. **36**(12): p. 2207-10.
237. Tomasi, G., et al., *Novel reference region model reveals increased microglial and reduced vascular binding of 11C-(R)-PK11195 in patients with Alzheimer's disease.* Journal of nuclear medicine : official publication, Society of Nuclear Medicine, 2008. **49**(8): p. 1249-56.
238. Schuitemaker, A., et al., *Evaluation of methods for generating parametric (R-[11C]PK11195 binding images.* Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism, 2007. **27**(9): p. 1603-15.
239. Cagnin, A., et al., *In-vivo measurement of activated microglia in dementia.* Lancet, 2001. **358**(9280): p. 461-7.

240. Yokokura, M., et al., *In vivo changes in microglial activation and amyloid deposits in brain regions with hypometabolism in Alzheimer's disease.* European journal of nuclear medicine and molecular imaging, 2011. **38**(2): p. 343-51.
241. Edison, P., et al., *Microglia, amyloid, and cognition in Alzheimer's disease: An [11C](R)PK11195-PET and [11C]PIB-PET study.* Neurobiol Dis, 2008. **32**(3): p. 412-9.
242. Okello, A., et al., *Microglial activation and amyloid deposition in mild cognitive impairment: a PET study.* Neurology, 2009. **72**(1): p. 56-62.
243. Wiley, C.A., et al., *Carbon 11-labeled Pittsburgh Compound B and carbon 11-labeled (R)-PK11195 positron emission tomographic imaging in Alzheimer disease.* Arch Neurol, 2009. **66**(1): p. 60-7.
244. Schuitemaker, A., et al., *Microglial activation in healthy aging.* Neurobiology of aging, 2010.
245. Yasuno, F., et al., *Increased binding of peripheral benzodiazepine receptor in Alzheimer's disease measured by positron emission tomography with [11C]DAA1106.* Biological psychiatry, 2008. **64**(10): p. 835-41.
246. Gulyas, B., et al., *Age and disease related changes in the translocator protein (TSPO) system in the human brain: positron emission tomography measurements with [11C]vinpocetine.* NeuroImage, 2011. **56**(3): p. 1111-21.
247. Owen, D.R., et al., *Two binding sites for [3H]PBR28 in human brain: implications for TSPO PET imaging of neuroinflammation.* Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism, 2010. **30**(9): p. 1608-18.
248. Fowler, J.S., et al., *Translational neuroimaging: positron emission tomography studies of monoamine oxidase.* Mol Imaging Biol, 2005. **7**(6): p. 377-87.
249. Saura, J., et al., *Molecular neuroanatomy of human monoamine oxidases A and B revealed by quantitative enzyme radioautography and in situ hybridization histochemistry.* Neuroscience, 1996. **70**(3): p. 755-74.
250. Levitt, P., J.E. Pintar, and X.O. Breakefield, *Immunocytochemical demonstration of monoamine oxidase B in brain astrocytes and serotonergic neurons.* Proc Natl Acad Sci U S A, 1982. **79**(20): p. 6385-9.
251. Jossan, S.S., et al., *Quantitative localization of human brain monoamine oxidase B by large section autoradiography using L-[3H]deprenyl.* Brain Res, 1991. **547**(1): p. 69-76.
252. Jossan, S.S., et al., *Visualization of brain monoamine oxidase B (MAO-B) in dementia of Alzheimer's type by means of large cryosection autoradiography: a pilot study.* Journal of neural transmission. Supplementum, 1990. **32**: p. 61-5.
253. Nakamura, S., et al., *Expression of monoamine oxidase B activity in astrocytes of senile plaques.* Acta Neuropathol (Berl), 1990. **80**(4): p. 419-25.
254. Santillo, A.F., et al., *In vivo imaging of astrocytosis in Alzheimer's disease: an (11)C-L: -deuteriodeprenyl and PIB PET study.* Eur J Nucl Med Mol Imaging, 2011.
255. Szardenings, K., et al., *Novel, small molecule [F18]-PET tracers for imaging of tau in human AD brains.* J Nucl Med, 2001. **52 (Supplement 1)**: p. 84.
256. Fodero-Tavoletti, M.T., et al., *18F-THK523: a novel in vivo tau imaging ligand for Alzheimer's disease.* Brain : a journal of neurology, 2011. **134**(Pt 4): p. 1089-100.
257. Mattsson, N., *CSF biomarkers in neurodegenerative diseases.* Clinical chemistry and laboratory medicine : CCLM / FESCC, 2011. **49**(3): p. 345-52.
258. Blennow, K. and H. Zetterberg, *Cerebrospinal fluid biomarkers for Alzheimer's disease.* Journal of Alzheimer's disease : JAD, 2009. **18**(2): p. 413-7.
259. Strozyk, D., et al., *CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study.* Neurology, 2003. **60**(4): p. 652-6.
260. Tapiola, T., et al., *Cerebrospinal fluid {beta}-amyloid 42 and tau proteins as biomarkers of Alzheimer-type pathologic changes in the brain.* Arch Neurol, 2009. **66**(3): p. 382-9.
261. Hesse, C., et al., *Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke.* Neurosci Lett, 2001. **297**(3): p. 187-90.
262. Ost, M., et al., *Initial CSF total tau correlates with 1-year outcome in patients with traumatic brain injury.* Neurology, 2006. **67**(9): p. 1600-4.
263. Zetterberg, H., et al., *Neurochemical aftermath of amateur boxing.* Arch Neurol, 2006. **63**(9): p. 1277-80.
264. Buerger, K., et al., *CSF phosphorylated tau protein correlates with neocortical neurofibrillary pathology in Alzheimer's disease.* Brain : a journal of neurology, 2006. **129**(Pt 11): p. 3035-41.
265. Shaw, L.M., et al., *Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects.* Ann Neurol, 2009. **65**(4): p. 403-13.

266. Riemenschneider, M., et al., *Cerebrospinal fluid tau and beta-amyloid 42 proteins identify Alzheimer disease in subjects with mild cognitive impairment.* Arch Neurol, 2002. **59**(11): p. 1729-34.
267. Johansson, P., et al., *Cerebrospinal fluid biomarkers for Alzheimer's disease: diagnostic performance in a homogeneous mono-center population.* Journal of Alzheimer's disease : JAD, 2011. **24**(3): p. 537-46.
268. Buchhave, P., et al., *Longitudinal study of CSF biomarkers in patients with Alzheimer's disease.* PloS one, 2009. **4**(7): p. e6294.
269. Bouwman, F.H., et al., *Longitudinal changes of CSF biomarkers in memory clinic patients.* Neurology, 2007. **69**(10): p. 1006-11.
270. Blennow, K., et al., *Longitudinal stability of CSF biomarkers in Alzheimer's disease.* Neurosci Lett, 2007. **419**(1): p. 18-22.
271. Andreasen, N., et al., *Sensitivity, specificity, and stability of CSF-tau in AD in a community-based patient sample.* Neurology, 1999. **53**(7): p. 1488-94.
272. Vemuri, P., et al., *Serial MRI and CSF biomarkers in normal aging, MCI, and AD.* Neurology, 2010. **75**(2): p. 143-51.
273. Hansson, O., et al., *Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study.* Lancet neurology, 2006. **5**(3): p. 228-34.
274. Visser, P.J., et al., *Prevalence and prognostic value of CSF markers of Alzheimer's disease pathology in patients with subjective cognitive impairment or mild cognitive impairment in the DESCRIPA study: a prospective cohort study.* Lancet neurology, 2009. **8**(7): p. 619-27.
275. Mattsson, N., et al., *CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment.* JAMA : the journal of the American Medical Association, 2009. **302**(4): p. 385-93.
276. Skoog, I., et al., *Cerebrospinal fluid beta-amyloid 42 is reduced before the onset of sporadic dementia: a population-based study in 85-year-olds.* Dement Geriatr Cogn Disord, 2003. **15**(3): p. 169-76.
277. Gustafson, D.R., et al., *Cerebrospinal fluid beta-amyloid 1-42 concentration may predict cognitive decline in older women.* J Neurol Neurosurg Psychiatry, 2007. **78**(5): p. 461-4.
278. Stomrud, E., et al., *Cerebrospinal fluid biomarkers predict decline in subjective cognitive function over 3 years in healthy elderly.* Dement Geriatr Cogn Disord, 2007. **24**(2): p. 118-24.
279. Fagan, A.M., et al., *Cerebrospinal fluid tau/beta-amyloid(42) ratio as a prediction of cognitive decline in nondemented older adults.* Arch Neurol, 2007. **64**(3): p. 343-9.
280. Fortea, J., et al., *Cerebrospinal fluid biomarkers in Alzheimer's disease families with PSEN1 mutations.* Neuro-degenerative diseases, 2011. **8**(4): p. 202-7.
281. Moonis, M., et al., *Familial Alzheimer disease: decreases in CSF Abeta42 levels precede cognitive decline.* Neurology, 2005. **65**(2): p. 323-5.
282. Ringman, J.M., et al., *Biochemical markers in persons with preclinical familial Alzheimer disease.* Neurology, 2008. **71**(2): p. 85-92.
283. Portelius, E., et al., *Distinct cerebrospinal fluid amyloid beta peptide signatures in sporadic and PSEN1 A431E-associated familial Alzheimer's disease.* Molecular neurodegeneration, 2010. **5**: p. 2.
284. Ringman, J.M., et al., *Longitudinal change in CSF biomarkers in a presymptomatic carrier of an APP mutation.* Neurology, 2011. **76**(24): p. 2124-5.
285. Mattsson, N., H. Zetterberg, and K. Blennow, *Lessons from Multicenter Studies on CSF Biomarkers for Alzheimer's Disease.* International journal of Alzheimer's disease, 2010. **2010**.
286. Mattsson, N., K. Blennow, and H. Zetterberg, *Inter-laboratory variation in cerebrospinal fluid biomarkers for Alzheimer's disease: united we stand, divided we fall.* Clinical chemistry and laboratory medicine : CCLM / FESCC, 2010. **48**(5): p. 603-7.
287. Mattsson, N., et al., *The Alzheimer's Association external quality control program for cerebrospinal fluid biomarkers.* Alzheimer's & dementia : the journal of the Alzheimer's Association, 2011. **7**(4): p. 386-395 e6.
288. Shaw, L.M., et al., *Qualification of the analytical and clinical performance of CSF biomarker analyses in ADNI.* Acta Neuropathol (Berl), 2011. **121**(5): p. 597-609.
289. Dubois, B., et al., *Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria.* Lancet neurology, 2007. **6**(8): p. 734-46.
290. de Jager, C.A., et al., *Retrospective evaluation of revised criteria for the diagnosis of Alzheimer's disease using a cohort with post-mortem diagnosis.* International journal of geriatric psychiatry, 2010. **25**(10): p. 988-97.

291. Oksengard, A.R., et al., *Lack of accuracy for the proposed 'Dubois criteria' in Alzheimer's disease: a validation study from the Swedish brain power initiative.* Dement Geriatr Cogn Disord, 2010. **30**(4): p. 374-80.
292. Jack, C.R., Jr., et al., *Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease.* Alzheimer's & dementia : the journal of the Alzheimer's Association, 2011. **7**(3): p. 257-62.
293. Sperling, R.A., et al., *Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease.* Alzheimer's & dementia : the journal of the Alzheimer's Association, 2011. **7**(3): p. 280-92.
294. Albert, M.S., et al., *The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease.* Alzheimer's & dementia : the journal of the Alzheimer's Association, 2011. **7**(3): p. 270-9.
295. McKhann, G.M., et al., *The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease.* Alzheimer's & dementia : the journal of the Alzheimer's Association, 2011. **7**(3): p. 263-9.
296. Jack, C.R., Jr., et al., *Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade.* Lancet Neurol, 2010. **9**(1): p. 119-28.
297. Nordberg, A., et al., *The use of PET in Alzheimer disease.* Nature reviews. Neurology, 2010. **6**(2): p. 78-87.
298. Ingelsson, M., et al., *Early Abeta accumulation and progressive synaptic loss, gliosis, and tangle formation in AD brain.* Neurology, 2004. **62**(6): p. 925-31.
299. Lo, R.Y., et al., *Longitudinal Change of Biomarkers in Cognitive Decline.* Arch Neurol, 2011.
300. Mueller, S.G., et al., *The Alzheimer's disease neuroimaging initiative.* Neuroimaging clinics of North America, 2005. **15**(4): p. 869-77, xi-xii.
301. Ellis, K.A., et al., *The Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging: methodology and baseline characteristics of 1112 individuals recruited for a longitudinal study of Alzheimer's disease.* Int Psychogeriatr, 2009. **21**(4): p. 672-87.
302. Weiner, M.W., et al., *The Alzheimer's disease neuroimaging initiative: progress report and future plans.* Alzheimer's & dementia : the journal of the Alzheimer's Association, 2010. **6**(3): p. 202-11 e7.
303. Caroli, A. and G.B. Frisoni, *The dynamics of Alzheimer's disease biomarkers in the Alzheimer's Disease Neuroimaging Initiative cohort.* Neurobiol Aging, 2010. **31**(8): p. 1263-74.
304. Waldemar, G., Y. Xu, and J. Mackell, *The effects of donezepil on dichotomous milestones in patients with Alzheimer's disease.* Int Psychogeriatr, 2009. **21**: p. 205.
305. Loy, C. and L. Schneider, *Galantamine for Alzheimer's disease and mild cognitive impairment.* Cochrane database of systematic reviews, 2006(1): p. CD001747.
306. Stefanova, E., et al., *Longitudinal PET evaluation of cerebral glucose metabolism in rivastigmine treated patients with mild Alzheimer's disease.* J Neural Transm, 2006. **113**(2): p. 205-18.
307. Tune, L., et al., *Donepezil HCl (E2020) maintains functional brain activity in patients with Alzheimer disease: results of a 24-week, double-blind, placebo-controlled study.* The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry, 2003. **11**(2): p. 169-77.
308. Ballard, C., et al., *Alzheimer's disease.* Lancet, 2011. **377**(9770): p. 1019-31.
309. Patlak, C.S., R.G. Blasberg, and J.D. Fenstermacher, *Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data.* J Cereb Blood Flow Metab, 1983. **3**(1): p. 1-7.
310. Minoshima, S., et al., *Preserved pontine glucose metabolism in Alzheimer disease: a reference region for functional brain image (PET) analysis.* J Comput Assist Tomogr, 1995. **19**(4): p. 541-7.
311. Lippa, C.F., et al., *Familial and sporadic Alzheimer's disease: neuropathology cannot exclude a final common pathway.* Neurology, 1996. **46**(2): p. 406-12.
312. Wold, S., et al., *Some recent developments in PLS modeling.* Chemometrics and Intelligent Laboratory Systems, 2001. **58**(2): p. 131-150.
313. Tzourio-Mazoyer, N., et al., *Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain.* Neuroimage, 2002. **15**(1): p. 273-89.
314. Bogdanovic, N. and J. Morris, *Neuropathological diagnostic criteria for brain banking*, in *Biomedical and Health Research Series*1995, IOS Press. p. 20-29.

315. Hammers, A., et al., *Three-dimensional maximum probability atlas of the human brain, with particular reference to the temporal lobe.* Hum Brain Mapp, 2003. **19**(4): p. 224-47.
316. Johansson, A., et al., *Evidence for astrocytosis in ALS demonstrated by [11C](L)-deprenyl-D2 PET.* J Neurol Sci, 2007. **255**(1-2): p. 17-22.
317. Hunt, A., et al., *Reduced cerebral glucose metabolism in patients at risk for Alzheimer's disease.* Psychiatry Res, 2007. **155**(2): p. 147-54.
318. Bassett, S.S., et al., *Familial risk for Alzheimer's disease alters fMRI activation patterns.* Brain : a journal of neurology, 2006. **129**(Pt 5): p. 1229-39.
319. Annoni, J.M., et al., *Chronic cognitive impairment following laterothalamic infarcts: a study of 9 cases.* Arch Neurol, 2003. **60**(10): p. 1439-43.
320. Villain, N., et al., *Relationships between hippocampal atrophy, white matter disruption, and gray matter hypometabolism in Alzheimer's disease.* The Journal of neuroscience : the official journal of the Society for Neuroscience, 2008. **28**(24): p. 6174-81.
321. Stenset, V., et al., *Diaschisis after thalamic stroke: a comparison of metabolic and structural changes in a patient with amnesic syndrome.* Acta neurologica Scandinavica. Supplementum, 2007. **187**: p. 68-71.
322. Aggleton, J.P. and M.W. Brown, *Episodic memory, amnesia, and the hippocampal-anterior thalamic axis.* The Behavioral and brain sciences, 1999. **22**(3): p. 425-44; discussion 444-89.
323. Pietrini, P., et al., *Pattern of cerebral metabolic interactions in a subject with isolated amnesia at risk for Alzheimer's disease: a longitudinal evaluation.* Dementia, 1993. **4**(2): p. 94-101.
324. Corder, E.H., et al., *Density profiles of Alzheimer disease regional brain pathology for the huddinge brain bank: pattern recognition emulates and expands upon Braak staging.* Exp Gerontol, 2000. **35**(6-7): p. 851-64.
325. Bogdanovic, N., et al., *APOE polymorphism and clinical duration determine regional neuropathology in Swedish APP(670, 671) mutation carriers: implications for late-onset Alzheimer's disease.* Journal of cellular and molecular medicine, 2002. **6**(2): p. 199-214.
326. Kadir, A., et al., *Positron emission tomography imaging and clinical progression in relation to molecular pathology in the first 11C-PIB PET Alzheimer patient.* Bain, 2010. **in press**.
327. Mega, M.S., et al., *Mapping histology to metabolism: coregistration of stained whole-brain sections to premortem PET in Alzheimer's disease.* Neuroimage, 1997. **5**(2): p. 147-53.
328. Vukovich, R., et al., *Brain metabolic correlates of cerebrospinal fluid beta-amyloid 42 and tau in Alzheimer's disease.* Dement Geriatr Cogn Disord, 2009. **27**(5): p. 474-80.
329. Mielke, R., et al., *Regional cerebral glucose metabolism and postmortem pathology in Alzheimer's disease.* Acta Neuropathol, 1996. **91**(2): p. 174-9.
330. Stenh, C., et al., *The Arctic mutation interferes with processing of the amyloid precursor protein.* Neuroreport, 2002. **13**(15): p. 1857-60.
331. Rabinovici, G.D. and W.J. Jagust, *Amyloid imaging in aging and dementia: testing the amyloid hypothesis in vivo.* Behavioural neurology, 2009. **21**(1): p. 117-28.
332. Jagust, W.J., et al., *Relationships between biomarkers in aging and dementia.* Neurology, 2009. **73**(15): p. 1193-9.
333. Forsberg, A., et al., *High PIB retention in Alzheimer's disease is an early event with complex relationship with CSF biomarkers and functional parameters.* Current Alzheimer research, 2010. **7**(1): p. 56-66.
334. Klunk, W.E., et al., *Binding of the positron emission tomography tracer Pittsburgh compound-B reflects the amount of amyloid-beta in Alzheimer's disease brain but not in transgenic mouse brain.* J Neurosci, 2005. **25**(46): p. 10598-606.
335. Klunk, W.E., et al., *The binding of 2-(4'-methylaminophenyl)benzothiazole to postmortem brain homogenates is dominated by the amyloid component.* J Neurosci, 2003. **23**(6): p. 2086-92.
336. Weigand, S.D., et al., *Transforming cerebrospinal fluid Abeta42 measures into calculated Pittsburgh Compound B units of brain Abeta amyloid.* Alzheimer's & dementia : the journal of the Alzheimer's Association, 2011. **7**(2): p. 133-41.
337. Okello, A., et al., *Conversion of amyloid positive and negative MCI to AD over 3 years: an 11C-PIB PET study.* Neurology, 2009. **73**(10): p. 754-60.
338. Verkhratsky, A., et al., *Astrocytes in Alzheimer's disease.* Neurotherapeutics, 2010. **7**(4): p. 399-412.
339. Terai, K., et al., *Apolipoprotein E deposition and astrogliosis are associated with maturation of beta-amyloid plaques in betaAPPswe transgenic mouse: Implications for the pathogenesis of Alzheimer's disease.* Brain Res, 2001. **900**(1): p. 48-56.
340. Smale, G., et al., *Evidence for apoptotic cell death in Alzheimer's disease.* Experimental neurology, 1995. **133**(2): p. 225-30.

341. Kuchibhotla, K.V., et al., *Synchronous hyperactivity and intercellular calcium waves in astrocytes in Alzheimer mice.* Science, 2009. **323**(5918): p. 1211-5.
342. Bateman, R.J., et al., *Autosomal-dominant Alzheimer's disease: a review and proposal for the prevention of Alzheimer's disease.* Alzheimer's research & therapy, 2011. **3**(1): p. 1.