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Joakim Hertze

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DOCTORAL DISSERTATION
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Title and subtitle

Pathophysiological Mechanisms and Diagnostic Markers in Alzheimer's Disease

Alzheimer's disease (AD), the most common cause of dementia, is a growing concern. As the life expectancy increases across the globe, the number of affected people is estimated to reach 100 million by 2050. Efforts to develop effective treatments for humans have mostly been based on the assumption that a faulty homeostasis of beta amyloid (Aβ) is the triggering or the driving factor behind this condition. So far these efforts have failed, making a better understanding of other pathophysiological mechanisms behind this disease necessary in order to find new therapeutic approaches. The aim for this thesis was to explore such mechanisms. Besides changes typical of AD, these patients often show signs of vascular disease. In order to explore how white matter changes (WMLs) affects the course of disease, we analyzed CSF levels of phosphorylated tau (P-tau) and assessed WMLs on brain scans in healthy controls and patients with mild cognitive impairment. We found that a pathological level of P-tau and WMLs in the parietal lobes independently increased the risk of developing AD dementia, but that the risk was considerably higher for patients that had both. Next, we analyzed the CSF/plasma ratio of albumin, as a proxy for blood-brain barrier integrity, and found it to be increased in AD and other common dementias. This ratio was not associated with the *APOE* genotype, as had previously been suggested, or markers of Aβ load. Instead, we found it to be associated with diabetes mellitus and markers of microvascular damage in the brain. We then moved on from vascular factors to other mechanisms suggested in AD.The insulin-like growth factor (IGF) related system is implicated in regulating life-span and in growth-promotion and neuroprotection in the human nervous system. There are reports of changes to the IGF-related system in cognitive disorders. When we analyzed components of this system in healthy controls and patients with AD we found differences in CSF and blood plasma levels of IGF-II and some of its associated binding proteins, possibly reflecting an activated general response to neuronal damage. Lastly, we analyzed CSF biomarkers of synaptic degeneration (neurogranin) and microglial activation (YKL-40) in healthy controls, patients with mild cognitive impairment and patients with common dementias. We found the former to be selectively increased in AD dementia, whereas the latter was increased in both AD and frontotemporal dementia. However, these biomarkers did not improve the diagnostic accuracy of either prodromal AD or AD dementia when compared to established CSF biomarkers of AD. Nevertheless, we think they are useful, since they reflect other processes in AD than Aβ deposition and axonal damage. We hope that the results from this thesis has provided new insights into the pathophysiological mechanisms in AD and that they might suggest novel routes for exploring this complex condition.

Key words: Alzheimer's disease, mild cognitive impairment, white matter lesions, brain reserve, blood-brain barrier, angiogenesis, neuroinflammation, neurogranin, YKL-40, VEGF, IGF, ICAM-1, VCAM-1

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To Anna, Alice and Alfred.

Nothing in life is as important as you think it is, while you are thinking about it.

— Daniel Kahneman

Contents

List of original papers

This thesis is based on the following original papers, referred to in the text as paper one, paper two, paper three and paper four:

One: Hertze, J., Palmqvist, S., Minthon, L., & Hansson, O. (2013). Tau Pathology and Parietal White Matter Lesions Have Independent but Synergistic Effects on Early Development of Alzheimer's Disease. *Dementia and Geriatric Cognitive Disorders Extra*, 3(1), 113–122.

Two: Janelidze S., Hertze J., Nägga K., Nilsson, C., Nilsson, K., the Swedish BioFINDER study, Wennström M., Hansson O. Increased blood-brain barrier permeability is associated with dementia and diabetes, but not amyloid pathology or APOE genotype. *Manuscript*

Three: Hertze, J., Nägga, K. N., Minthon, L., & Hansson, O. (2014). Changes in cerebrospinal fluid and blood plasma levels of IGF-II and its binding proteins in Alzheimer's disease: an observational study. *BMC Neurology*, 14(1), 1–8.

Fyra: Janelidze, S., Hertze, J., Zetterberg, H., Landqvist Waldö, M., Santillo, A., Blennow, K. & Hansson, O. (2016). Cerebrospinal fluid neurogranin and YKL-40 as biomarkers of Alzheimer's disease. *Annals of clinical and translational neurology*, 3(1), 12–20.

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Original papers not included in this thesis

Hölttä, M., Hansson, O., Andreasson, U., Hertze, J., Minthon, L., Nägga, K., et al. (2013). Evaluating amyloid-β oligomers in cerebrospinal fluid as a biomarker for Alzheimer's disease. (S. T. Ferreira, Ed.) *PLoS ONE*, 8(6), e66381.

Olsson, B., Hertze, J., Ohlsson, M., Nägga, K., Höglund, K., Basun, H., et al. (2012). Cerebrospinal Fluid Levels of Heart Fatty Acid Binding Protein are Elevated Prodromally in Alzheimer's Disease and Vascular Dementia. *Journal of Alzheimer's Disease : JAD*, 34(3), 673-679.

Olsson, B., Hertze, J., Lautner, R., Zetterberg, H., Nägga, K., Höglund, K., Basun, H., et al. (2012). Microglial Markers are Elevated in the Prodromal Phase of Alzheimer's Disease and Vascular Dementia. *Journal of Alzheimer's disease : JAD*, 33(1), 45-53.

Palmqvist, S., Hertze, J., Minthon, L., Wattmo, C., Zetterberg, H., Blennow, K., Londos, E., et al. (2012). Comparison of Brief Cognitive Tests and CSF Biomarkers in Predicting Alzheimer's Disease in Mild Cognitive Impairment: Six-Year Follow-Up Study. (J. C. S. Breitner, Ed.) *PLoS ONE*, 7(6), e38639.

Hertze, J., Minthon, L., Zetterberg, H., Vanmechelen, E., Blennow, K., & Hansson, O. (2010). Evaluation of CSF biomarkers as predictors of Alzheimer's disease: a clinical follow-up study of 4.7 years. *Journal of Alzheimer's disease : JAD*, 21(4), 1119–1128.

Abstract

Alzheimer's disease (AD), the most common cause of dementia, is a growing concern. As the life expectancy increases across the globe, the number of affected people is estimated to reach 100 million by 2050. Efforts to develop effective treatments for humans have mostly been based on the assumption that a faulty homeostasis of beta amyloid (Aβ) is the triggering or the driving factor behind this condition. So far these efforts have failed, making a better understanding of other pathophysiological mechanisms behind this disease necessary in order to find new therapeutic approaches. The aim for this thesis was to explore such mechanisms.

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Next, we analyzed the CSF/plasma ratio of albumin, as a proxy for blood-brain barrier integrity, and found it to be increased in AD and other common dementias. This ratio was not associated with the *APOE* genotype, as had previously been suggested, or markers of Aβ load. Instead, we found it to be associated with diabetes mellitus and markers of microvascular damage in the brain.

We then moved on from vascular factors to other mechanisms suggested in AD.

The insulin-like growth factor (IGF) related system is implicated in regulating life span and in growth-promotion and neuroprotection in the human nervous system. There are reports of changes to the IGF-related system in cognitive disorders. When we analyzed components of this system in healthy controls and patients with AD we found differences in CSF and blood plasma levels of IGF-II and some of its associated binding proteins, possibly reflecting an activated general response to neuronal damage.

Lastly, we analyzed CSF biomarkers of synaptic degeneration (neurogranin) and microglial activation (YKL-40) in healthy controls, patients with mild cognitive impairment and patients with common dementias. We found the former to be selectively increased in AD dementia, whereas the latter was increased in both AD and frontotemporal dementia. However, these biomarkers did not improve the

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We hope that the results from this thesis have provided new insights into the pathophysiological mechanisms in AD and that they might suggest novel routes for exploring this complex condition.

Populärvetenskaplig sammanfattning på svenska

Alzheimers sjukdom är den vanligaste orsaken till uppkomsten av demens. Detta tillstånd innebär att delar av hjärnan skrumpnar på ett karakteristiskt vis, när nervceller går under. Den drabbade brukar först märka svårigheter med korttidsminnet, för att senare drabbas av språksvårigheter, räknesvårigheter, svårigheter med rumslig uppfattning och svårigheter att veta hur man ska utföra vardagliga aktiviteter. Efter många års sjukdom brukar hela storhjärnans funktion vara nedsatt. Ålder och i viss mån ärftlighet är de viktigaste riskfaktorerna. I takt med att medellivslängden ökar i världen kommer allt fler att drabbas av detta tillstånd, vilket gör att behovet av effektiva behandlingar är överhängande. Under de senaste två årtiondena har de flesta ansträngningarna att ta fram sådana behandlingar sprungit ur observationen att Alzheimersjuka har ansamlingar av sjuklig betaamyloid (plack) i storhjärnans bark. Trots lovande resultat från djurmodeller har man hittills inte lyckats att ta fram läkemedel för människor som påverkar bildandet av denna sjukliga betaamyloid, eller som minskar mängden som ansamlats.

Den Alzheimersjuka hjärnan uppvisar även andra förändringar, som förlust av kontaktpunkter (synapser) mellan nervceller, inflammatoriska förändringar, ärrbildning, förändringar i hjärnans vita substans och ansamlingar av andra ämnen, som nystan av äggviteämnet tau. Eftersom betaamyloidspåret hittills inte varit fruktbart finns ett behov av att undersöka andra möjliga mekanismer bakom Alzheimers sjukdom, i hopp om att hitta lovande ingångsvinklar för behandling. Målet för denna avhandling var just att närmare undersöka några av dessa mekanismer.

Sedan tidigare vet man att Alzheimersjuka inte bara har karakteristiska förändringar som plack och nystan i sina hjärnor. Med röntgenundersökningar som datortomografi eller magnetresonanstomografi ser man ofta tecken till kärlförändringar, som rester efter gamla infarkter, eller vitsubstansförändringar. Just vitsubstansförändringar är något man ofta ser även hos friska äldre.

Vi ville studera hur vitsubstansförändringar påverkar sjukdomsförloppet hos patienter med lindriga minnesbesvär, som vi vet löper risk att utveckla Alzheimers sjukdom. Vi undersökte därför ryggvätska med avseende på en indikator för Alzheimerförändringar (sjukligt förhöjd nivå av äggviteämnet P-tau) och bedömde

röntgenbilder med avseende på förekomst av vitsubstansförändringar hos friska kontroller och patienter med lindriga minnesbesvär. Vi kunde då se att för hög nivå av P-tau och vitsubstansförändringar i tinningloberna var för sig ökade risken för dessa patienter att utveckla Alzheimers sjukdom, men att risken var betydligt större om man hade båda förändringarna.

Vi gick sedan vidare och undersökte ett annat fenomen som vanligen observeras vid kärldemenser. En fullt frisk människa har en barriär mellan blodomloppet och hjärnvävnaden (blod-hjärnbarriären), som skyddar den känsliga hjärnans miljö. Vid kärldemenser observerar man vanligen att denna barriär luckras upp, vilket kan få negativa konsekvenser. Vi ville nu undersöka blod-hjärnbarriärens funktion vid Alzheimers sjukdom, samt vid andra vanliga demensformer. Vi ville också se om vi kunde identifiera riskfaktorer för en sådan påverkad funktion. Vi såg i vårt material indirekta tecken till uppluckring av blod-hjärnbarriären både vid kärldemens och Alzheimers sjukdom, men även vid andra vanliga demensformer. Denna uppluckring kunde inte kopplas till förekomsten av en eller flera *APOE* ε4 gener, som tidigare föreslagits, eller till mängden ansamlat betaamyloid i hjärnan. Istället kunde vi se ett samband med förekomst av diabetes mellitus och med indirekta tecken till skador i hjärnans småkärl.

För de två sista delarbetena lämnade vi de kärlrelaterade faktorerna, för att undersöka andra föreslagna mekanismer vid Alzheimers sjukdom.

Systemet med insulin-liknande tillväxtfaktor (IGF) har beskrivits vara påverkat vid olika demenstillstånd. Detta system anses vara viktigt bland annat för att reglera livslängd och för att stimulera tillväxt och ge skydd i det mänskliga nervsystemet. I vårt material såg vi förändringar i detta system hos patienter med Alzheimers sjukdom, framförallt i form sänkta nivåer av hormonet IGF-II i blod och ökade nivåer i ryggvätska, vilket kan spegla en ökad aktivitet i hjärnans försvar mot nervcellsundergång.

Slutligen undersökte vi en markör i ryggvätska för synapssönderfall (neurogranin) och en markör i ryggvätska för neuroinflammation (YKL-40) i förhoppningen att dessa skulle kunna öka möjligheterna för en tidig diagnos av Alzheimers sjukdom. Dessvärre var dessa båda markörer inte bättre på att förutse kommande sjukdom, eller på att skilja Alzheimers sjukdom från andra demenstillstånd, när vi jämförde med markörer som redan idag används inom sjukvården. Vi anser ändå att dessa markörer har sin plats, då de speglar andra processer än just ansamling av betaamyloid i hjärnan.

Sammanfattningsvis har delarbetena i denna avhandling belyst hur vitsubstansförändringar och typiska Alzheimerförändringar samverkar i sjukdomsprocessen, hur blod-hjärnbarriärens funktion påverkas vid vanliga demenstillstånd, hur IGF-systemet förändras vid Alzheimers sjukdom, samt värdet av markörer för synapssönderfall och neuroinflammation vid diagnostiken av Alzheimers sjukdom. Vi hoppas att våra fynd bidrar till en ökad förståelse för mekanismerna bakom

denna folksjukdom, vilket i förlängningen förhoppningsvis leder till effektiva behandlingar.

Abbreviations

AA	Alzheimer's Association
AD	Alzheimer's disease
ADRDA	Alzheimer's Disease and Related Disorders Association
APOE	Apolipoprotein E
APP	Amyloid precursor protein
Aβ	Beta amyloid
Aβ38	Beta amyloid 1–38
Aβ40	Beta amyloid 1–40
Aβ42	Beta amyloid 1–42
BBB	Blood-brain barrier
CSF	Cerebrospinal fluid
DLB	Dementia with Lewy-bodies
FTD	Frontotemporal dementia
ICAM-1	Intercellular cell adhesion molecule–1
IGF	Insulin-like growth factor
IGF-I	Insulin-like growth factor one
IGF-II	Insulin-like growth factor two

IGFBP-2	IGF-binding protein 2
IGFBP-3	IGF-binding protein 3
IR	Insulin receptor
MCI-AD	Prodromal AD, patient who progressed from mild cognitive impairment to Alzheimer's disease
MCI	Mild cognitive impairment
NFT	Neurofibrillary tangle
NIA	National Institute on Aging
NINCDS	National Institute of Neurological and Communicative Disorders and Stroke
P-tau	Phosphorylated tau
PDD	Parkinson's disease with dementia
PET	Positron emission tomography
PlGF	Placental growth factor
SCD	Subjective cognitive decline
sMCI	Stable MCI
SUVR	Standardized uptake value ratio, defined as the uptake in a volume of interest (VOI), normalized for the cerebellar cortex uptake.
VaD	Vascular dementia
VCAM-1	Vascular cell adhesion molecule–1
VEGF	Vascular endothelial growth factor
WML	White matter lesion

Introduction

Dementia

Dementia, or major neurocognitive disorder, is a heterogeneous group of illnesses affecting a wide variety of cognitive and emotional functions. They not only cause immense human suffering, but also have a major economic impact on society as a whole (Comas-Herrera et al., 2003; Lowin et al., 2001). The number of people affected has been estimated to double every 20 years, making better diagnostic methods and treatments an ever growing concern (Ferri et al., 2005).

The term dementia is used to label conditions with a decline in one or more cognitive domains to the point where it profoundly impacts the ability to lead an independent life. The cognitive impairment must not only be present during episodes of delirium and traditionally the symptoms should not be better explained by a major depressive episode or schizophrenia. Generally, the term dementia should be reserved for conditions that are non-reversible in nature.

Many conditions can lead to a syndrome of dementia. They are often divided into two main groups: the vascular dementias and the neurodegenerative dementias. Of the latter, Alzheimer's disease (AD) is the most common, in all likelihood comprising 50 to 60 percent of all dementias. Also dementias with Lewy bodies (Lewy body dementia, DLB; and Parkinson's disease with dementia, PDD) belong to this group, as do the frontotemporal dementias (the behavioral variant, bvFTD; the semantic variant; the progressive non-fluent aphasia; and the motorneuron variant). The vascular dementias (VaD) can in turn roughly be divided into cortical vascular dementia (multi-infarct dementia) and subcortical vascular dementia. There are also syndromes secondary to strategic infarctions, cerebral hemorrhaging and diseases of the blood vessels in the brain, such as angitis and cerebral amyloid angiopathy. Many, if not most, patients have a mix of different conditions (Wahlund et al., 2011).

Mild cognitive impairment

Between cognitive changes that are a part of normal aging and those of dementia is a condition most commonly referred to as mild cognitive impairment, or MCI. Here, the ability to lead an independent life is preserved, even though the affected person might have to deploy new strategies to compensate for difficulties. In 2004, Petersen et al. suggested the following algorithm to help in the evaluation of cognitive complaints:

1. A patient with a cognitive complaint…
2. …who is neither cognitively normal, nor demented…
3. …and where the clinician finds evidence of a cognitive decline from patient history and preferably a collateral informant…
4. and where the functional decline as a result of that cognitive decline is not significant…
5. …could be diagnosed as having MCI.

The MCI syndrome can be further sub classified as amnestic, single non-memory domain, or involving multiple domains (Petersen, 2004; Winblad et al., 2004).

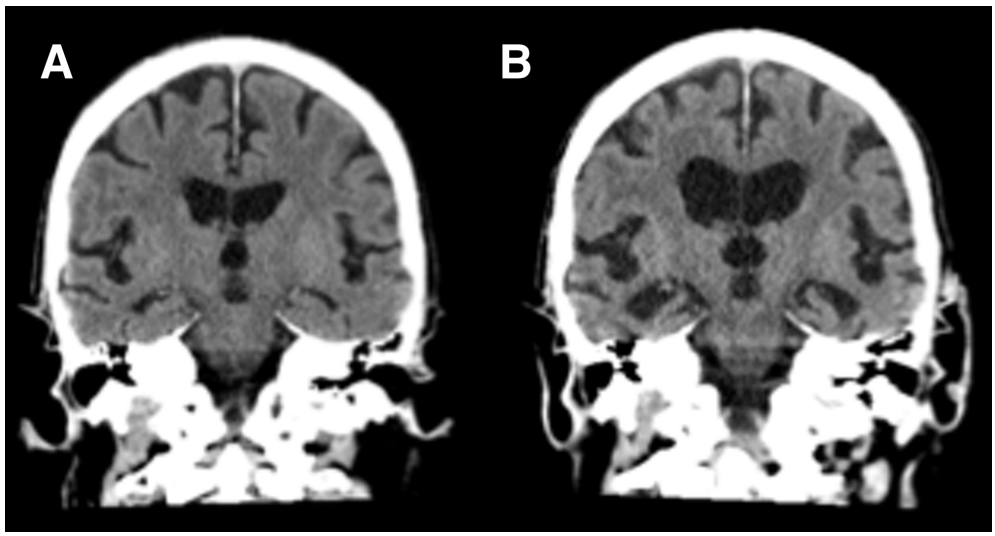
While all patients with dementia at some point have progressed through a stage of MCI, not all patients with MCI will develop dementia. While the risk of developing AD (or other dementias) is greater than in a person who is cognitively healthy, some MCI patients are cognitively stable over time, or even improve their cognitive functioning (Winblad et al., 2004). It has been estimated that between 10 and 20 percent of patients with MCI due to underlying AD pathology progress to dementia each year (Petersen et al., 2010).

Alzheimer's disease as a clinical entity

AD is a disorder characterized by typical regional atrophy of the brain, together with plaques and neurofibrillary tangles in the cerebral cortex. Usually, the affected patient first notices difficulties with episodic memory, then dysphasia of the word-finding type, dyscalculia, dyspraxia, difficulties with visuospatial abilities and executive difficulties. As the condition progresses, impairment in motor function, dystonia, myoclonia and epileptic seizures may further complicate the deteriorating cognitive functions. These symptoms mirror the progression of the disease, generally thought to start in the medial temporal lobe, in the hippocampus and para-hippocampal structures, before it spreads to the parietal lobes and then to the

entire cerebral cortex (Wahlund et al., 2011). Known risk factors for AD include age, factors related to cognitive reserve, vascular risk factors, and genetic factors, such as mutations of the amyloid precursor protein (*APP*) gene, the presenilin 1 and 2 gene, and carrying the ε4 allele of apolipoprotein E (*APOE*) (Blennow et al., 2006).

In reality, AD is likely to be many different diseases, with clinical symptoms and neuropathological changes in common. It is useful to differentiate between AD as a clinical syndrome and AD as typical neuropathological changes, or biomarkers considered to reflect those changes. It has been suggested to denote the former AD-C (AD-clinical) and the latter as AD-P (AD-pathophysiological) (Sperling et al., 2011). In a clinical context, we differentiate between early-onset AD and late-onset AD.



CT scans from a patient with AD, progressing from A to B during a timespan of three years.

Neuropathology in AD

In 2012, the National Institute on Aging and Alzheimer's Association (NIA-AA) proposed updated guidelines for the neuropathological assessment of AD. These guidelines differ in a few ways from the earlier criteria (the NIA-Reagan criteria), most notably in no longer requiring a clinical diagnosis of dementia (Hyman et al., 2012).

Of the several characteristic lesions seen in AD, senile plaques and neurofibrillary tangles (NTFs) are considered essential for the diagnosis.

Senile plaques are extracellular deposits of amyloid beta (Aβ) peptides, with a complex nomenclature and morphology. Aβ deposits can be at the center of a cluster of dystrophic neurites that often (but not always) contain hyperphosphorylated tau. These are commonly called neuritic plaques. Aβ deposits can also be organized into other structures, such as diffuse plaques, cotton-wool plaques, amyloid lakes and subpial bands. Of all the different forms of Aβ deposits, neuritic plaques are considered to be most closely related to neuronal injury and consequently with clinical symptoms (Hyman et al., 2012).

Neuropathologists score Aβ plaques according to criteria modified from Thal et al (Thal et al., 2002), while neuritic plaques are scored according to criteria modified from Consortium to Establish a Registry for Alzheimer's Disease (CERAD) (Mirra et al., 1991).

NTFs are, at least in early stages, intraneuronal fibrils primarily composed of abnormally phosphorylated tau (P-tau). They can be visualized with a variety of histochemical stains and with immunohistochemistry directly targeting tau or P-tau epitopes. NTFs are commonly observed in the limbic regions early in the disease, but later on also in other regions, such as association areas of the cortex, some subcortical nuclei and in brainstem regions (Hyman et al., 2012). NTFs are found to correlate with clinical symptoms in AD (Bennett et al., 2004; Gómez-Isla et al., 1997; Lace et al., 2009). The accumulation of NTFs are described using a staging scheme suggested by Braak and Braak in 1991 (Braak & Braak, 1991).

Other features of neuropathological changes in AD are more difficult to assess by conventional histopathological methods, or are considered to be less closely related to upstream events of the disease. These include synapse loss, neuron loss, atrophy, gliosis, degenerative white matter changes, granulovacuolar degeneration, cerebral amyloid angiopathy and other protein aggregates, such as TDP-43, Lewy bodies and actin-immunoreactive Hirano bodies (Hyman et al., 2012).

The genetics of Alzheimer's disease

Familial Alzheimer's disease is thought to only represent up to one percent of all disease cases (Wahlund et al., 2011). It is an autosomal dominant disorder with onset before 65 years. While there are several known mutations of the *APP* gene on chromosome 21, these have only been demonstrated in a few disease cases. Most instances of these hereditary forms are instead explained by mutations in the highly homogenous presenelin 1 (*PSEN1*) and presenelin 2 (*PSEN2*) genes (Blennow et al., 2006).

Evidence suggests that the *APOE* ε4 allele accounts for most of the genetic risk in sporadic AD (Raber et al., 2004) and the predictive value of *APOE* ε4 homozygosity is as high as for many other Mendelian diseases (Hampel et al., 2010). It has been identified as a major susceptibility gene for familial AD as well (Huang & Mahley, 2014; Blennow et al., 2006).

Human ApoE is a lipid protein consisting of 299 amino acids with two structural domains. The N-terminal domain contains the receptor-binding region and the C-terminal domain contains the lipid-binding region. The gene is located on chromosome 19q13 and exists in three major variants, *APOE* ε2, *APOE* ε3 and *APOE* ε4, which in turn encode three major isoforms of the molecule, ApoE2, ApoE3 and ApoE4. The differences among the three isoforms are only found in position 112 and 158 in the amino acid sequence, which have a profound effect on the structure and function of ApoE (Yu et al., 2014).

ApoE is the predominant apolipoprotein in the brain and is a part of the transport system for lipids in the cells of different organs and tissues. It plays a crucial role in regulating the clearance of lipoproteins from the plasma by serving as the ligand for binding to specific cell-surface receptors. It also appears to play an important roll in neuronal repair by redistributing lipids to regenerating axons and Schwann cells during remyelinisation. ApoE also serves as the main lipid transportation vehicle in the cerebrospinal fluid (CSF) (Huang & Mahley, 2014).

ApoE3 is thought to be the normal form of this molecule (Huang & Mahley, 2014). ApoE2 has been found to be defective in lipoprotein receptor-binding and is associated with the genetic disorder type III hyperlipoproteinemia (Mahley et al., 1999). It may also be a risk factor for developing diabetes nephropathy (Araki, 2014). ApoE4 seems to be less efficient than other variants in reusing membrane lipids in neuronal repair (Poirier, 1994). It has also been suggested to be involved in Aβ deposition, by promoting Aβ fibrillisation and plaque formation (Holtzman et al., 2000). However, it seems Aβ does not directly interact with ApoE to a significant extent. Instead, Aβ competes with ApoE for binding to the lipoprotein receptor-related protein 1 (LRP1) in a concentration-dependent manner, which likely impacts its clearance by glia cells and transport across the blood-brain barrier (Verghese et al., 2013). The equilibrium that exists between Aβ in plasma and in the CSF is the basis for the "peripheral sink" hypothesis of AD treatment, which favors clearing peripheral Aβ species in order to transport Aβ out of the CSF (Wildsmith et al., 2013).

In 1993 Corder at al. used genetic analysis to identify *APOE* ε4 as a major risk factor for AD (Corder et al., 1993). A short time after *APOE* ε2 was found to "protect" against disease (Chartier-Harlin et al., 1994). One *APOE* ε4 allele seemingly shifts the risk curve in time, advancing the onset of the disease by five years, while two *APOE* ε4 alleles advances the onset by ten years. One copy of the *APOE* ε2 allele instead postpones the onset of the disease by five years.

It might be useful to divide individuals at risk of developing AD into three groups, based on their *APOE* ε4 status. The first group would include individuals with two alleles and a resulting high risk of AD. Other genes and the polymorphism of the APOE promoter further modulate this risk. The second group would include individuals who have one allele and therefore have a moderate risk of AD. In this group, the risk is modulated by other *APOE* alleles (ε2 being less risky than ε3) and by the polymorphism of the *APOE* promoter. The third group would include individuals with no *APOE* ε4 allele and who therefore are at low risk of AD (Hampel et al., 2010).

The amyloid cascade hypothesis

The so-called amyloid cascade hypothesis proposes that a skewed amyloid homeostasis promotes the accumulation of amyloid beta 1-42 (Aβ42) in the brain. This is commonly thought to represent the earliest event in the development of AD, ultimately leading to neuronal death and dementia. This theory is supported by the fact that familial forms of AD carry mutations in both the substrate (APP) and a key enzyme (presenilin) for Aβ production. Also, individuals with Down's syndrome, who carry an extra copy of chromosome 21 where the *APP* gene is located, generally develop Aβ plaques early in life (Blennow et al., 2006). The recent discovery of a "protective" gene mutation (A673T), adjacent to the aspartyl protease beta-site in the *APP*-gene, also lends support to this hypothesis. This mutation has been found to be associated with a lower prevalence of AD in several large cohorts, but also with less cognitive decline in cognitively healthy older individuals (Jonsson et al., 2012).

APP is a transmembrane protein, existing in different isoforms. It can be processed along two main pathways. In the α-secretase pathway, α-secretase cleaves APP within the Aβ domain, which releases large soluble APP fragment (α-sAPP) and thereby prevents the generation of Aβ. In the β-secretase pathway, β-secretase cleaves APP just before the Aβ domain, releasing soluble βsAPP. The remaining C-terminal fragment is then cleaved by the γ-secretase complex, releasing free Aβ peptides, most commonly with a length of 40 or 42 amino acids. Most β-secretase activity originates from the protease β-site APP-cleaving enzyme 1 (BACE1) (Vassar et al., 1999). Proteases belonging to the ADAM family of disintegrin and metalloproteases have been identified in the α-secretase pathway (Lammich et al., 1999). γ-secretase is an intramembranous protease complex, in which presenilin constitutes the active site (Gandy, 2005).

Aβ is continuously produced in normal cell metabolism in the central nervous system (CNS) (Selkoe, 2006). Under normal conditions, brain Aβ is degraded by the peptidases insulin-degrading enzyme, neprilysin and by endothelin-converting

enzyme. Aβ is also cleared from the brain in a process balanced by the active transport of Aβ across the blood-brain barrier by low-density lipoprotein receptor (LDLR) family members (Wildsmith et al., 2013).

Initially, only Aβ in insoluble plaques was assumed to be neurotoxic, but newer findings suggest that soluble Aβ oligomers might be the culprits instead, inhibiting hippocampal long-term potentiation, removing synaptic glutamate receptors and eliminating glutamate synapses (Zetterberg et al., 2010; Blennow et al., 2006; Walsh & Selkoe, 2004).

In a stereotypical manner, Aβ seems to accumulate in brain regions that are considered part of specific brain networks, primary the default mode network (Buckner et al., 2005). It also deposits in other regions of high connectivity, commonly called cortical hubs, which are part of other intrinsic networks (Buckner et al., 2009). Older individuals with evidence of amyloid accumulation on PET (positron emission tomography) imaging have demonstrated impaired ability to modulate activity in the default mode network. In particular, failure to activate the posterior cingulate/precuneus regions during episodic memory encoding tasks have been associated with elevated Aβ accumulation in these regions (Sperling et al., 2014).

Hyperphosphorylation of tau

The NTFs present in AD are composed of abnormally phosphorylated tau-protein (Grundke-Iqbal et al., 1986). Tau is an axonal protein that binds microtubules and promotes their assembly and stability. Tau-phosphorylation is regulated by a balance between multiple kinases and phosphatases. The hyper-phosphorylation seen in AD starts intracellularly and leads to the sequestration of normal tau and other microtubule-associated proteins, which in turn leads to breakdown of microtubules and a resulting impaired axonal transport (Iqbal et al., 2005). Tau also tends to aggregate into insoluble fibrils in tangles, further compromising neuronal function. Tau pathology starts in transentorhinal neurons and then spreads to the hippocampus, amygdala and finally to neocortical association areas (Braak et al., 1999). Recent preliminary data from tau-PET studies suggest that medial temporal lobe accumulation of tau is very common after the age of 60 (Sperling et al., 2014). Some evidence suggests that Aβ triggers imbalanced activities of protein kinases and phosphatases, which in turn affect the phosphorylated state of the brain. This might prove to be the link between the two major neuropathological findings seen in AD (Oliveira et al., 2015).

Inflammation, angiogenesis and microvascular pathology

A number of risk factors for vascular disease, as well as biomarkers for systemic inflammation and microvascular pathology, have been associated with AD (Hall et al., 2013).

The inflammation observed in AD is characterized by activated microglia cells that surround Aβ plaques. These microglia cells are involved in clearing Aβ deposits by phagocytic activity, but also in producing pro-inflammatory cytokines (such as interleukins and tumor necrosis factor-α) and reactive oxygen and nitrogen species, which all cause neuronal damage. The damaged neurons then release signals that can overactivate microglia and induce a cycle of neuronal damage in a process known as reactive microglios. The mechanisms through which Aβ plaques initiate an inflammatory response is not fully known (Doens & Fernández, 2014).

A recent metaanalysis suggested a 28 percent reduction in risk of incident AD for users of non-steroid anti-inflammatory drugs (NSAIDs), with greater benefits from long-term use rather than short-term use. Suppression of microglial activation is one possible explanation for this effect. However, this meta-analysis was mainly based on observational data. The only randomized controlled trial included in this analysis failed to show any benefit of this class of drugs, which is disconcerting (Wang et al., 2015).

Matrix metalloproteinases (MMPs) are considered to play an important role in the neuroinflammation seen in AD. Gelatinases, such as MMP-2 and MMP-9, degrade molecules in the basal lamina around capillaries, enable angiogenesis and neurogenesis, participate in inducing cell death and play an important role in injury and repair. Stromelysins, such as MMP-3, MMP-10 and MMP-11, metabolize components of the extracellular matrix, while collagenases, such as MMP-1, MMP-8, MMP-13 and MMP-18, degrade fibrillar collagen in bone and cartilage (Wang et al., 2014). The activity of MMPs is regulated by specific tissue inhibitors (TIMPs), which also promote cell proliferation and synaptic plasticity and are hypothesized to have anti-apoptotic activity. (Mroczko et al., 2014).

YKL-40 (chitinase-3-like protein 1) is a glycoprotein considered to be a marker for macrophage and microglial differentiation and activation (Hellwig et al., 2015) and thus reflects ongoing inflammation in a variety of human diseases (Bonneh-Barkay et al., 2008; 2010; Comabella et al., 2010; Kjaergaard et al., 2015). CSF-levels of YKL-40 appear to be elevated in AD, VaD and frontotemporal dementia (FTD) (Alcolea et al., 2014; Olsson et al., 2012), but not in Parkinson's disease or DLB (Wennström et al., 2015). This protein is also increased in normal aging and preclinical AD (Alcolea et al., 2015; Sutphen et al., 2015).

Risk factors for vascular disease, as well as vascular disease in itself, is strongly associated with AD (Breteler, 2000). While some authors see vascular

factors as intrinsically linked to the AD disease process, others argue that vascular pathology occurs independently and that it simply increases the probability of dementia symptoms in patients with otherwise asymptomatic low-grade AD pathology (Riekse et al., 2004; Snowdon et al., 1997). In AD, the blood vessels of the brain commonly show angiopathy, with deposited Aβ consisting of 40 amino acids (Aβ40) (Ellis et al., 1996). Carrying two alleles of *APOE* ε4 is not only correlated with an increased risk of sporadic AD, but there is also a strong association between vascular pathology and the *APOE* ε4 genotype, suggesting microvascular damage to be a part of the pathogenesis of AD (Zipser et al., 2007). Previous studies suggest a reciprocal relationship between AD-pathology and ischemic, or even hypoxic, events in AD (Pimentel-Coelho & Rivest, 2012).

The neurovascular hypothesis suggests that dysfunctional blood vessels impair delivery of nutrients to neurons and reduce the clearance of Aβ from the brain, thereby contributing to cognitive disease (Iadecola, 2004). There is also data that indicates an impaired angiogenesis in patients with AD (Pimentel-Coelho & Rivest, 2012).

There is strong evidence of decreased vascular density in AD. Reduced capillary density in this disease may in part be due to a reduction in angiogenesis caused by the vascular endothelial growth factor (VEGF) becoming bound to Aβ and then sequestered in senile plaques. This leads to a failure in vascular recovery from hypoxia-induced bouts of capillary loss and plays a part in the reduction of vascular and functional reserve (Brown & Thore, 2011). VEGF—in reality a family of several known growth factors, including placental growth factor (PlGF)—is viewed as one of the key regulators of angiogenesis (Roskoski, 2008).

Hunter et al. found neuropathological evidence of a concomitant loss of functional capillaries and brain volume in AD subjects. They also demonstrated a trend of decreasing vesicular acetylcholine-transporter staining, a marker for cortical cholinergic afferents that contribute to arteriolar vasoregulation (Hunter et al., 2012). A study by Cheung et al found that patients with AD were more likely to have structural changes (narrower retinal venules, sparser and more tortuous retinal microvascular network) in the retinal microvasculature network of the eye, possibly reflecting similar changes in the cerebral microcirculation (Cheung et al., 2014).

Among a set of soluble adhesion molecules used as biomarkers for microvascular pathology are vascular cell adhesion molecule-1 (VCAM-1) and intercellular cell adhesion molecule-1 (ICAM-1). These two biomarkers have been linked to the development of AD, as well as late-life depression (Hall et al., 2013).

Alzheimer's disease as a neurodegenerative metabolic disorder

The insulin-like growth factor (IGF) related system consists of two ligands, IGF-I and IGF-II, their binding proteins and their cell-surface receptors. Some authors also include insulin and the insulin receptor (IR) with this system (de la Monte, 2009). While IRs are widely distributed throughout the brain, the hippocampus presents particularly high levels of these receptors (De Felice, 2013). Both IGF-I and IGF-II bind to the IR, in addition to their own receptors, IGF-IR and IGF-IIR (Russo et al., 2005).

Both insulin and IGF modulate neuronal growth and survival, differentiation, migration, metabolism, gene expression, protein synthesis, cytoskeletal assembly, synapse formation and plasticity (D'Ercole et al., 1996). Impaired signaling through insulin and IGF receptors adversely affects a broad range of neuronal and glial functioning, including glucose homeostasis, energy metabolism and white matter fiber structure and function. While systemic insulin (and IGF) can enter the CNS from the bloodstream, locally produced insulin seems more critical for responding to the immediate needs associated with learning and memory (D'Ercole et al., 1996; Rivera et al., 2005).

Circulating levels of IGF-I and IGF-II fall progressively as we grow older (Garcia-Fernandez et al., 2011; van Dam & Aleman, 2004) and an association between levels of IGF-I and cognitive decline has been described (Watanabe et al., 2005; van Dam et al., 2000; Aleman & Torres-Alemán, 2009). Lower circulating levels of IGF-I in blood plasma have also been found to increase the risk of postoperative cognitive dysfunction in elderly patients (Jiang et al., 2015).

Researchers have found evidence suggesting that the brains of patients with AD are resistant to insulin and IGF, while they also have lower levels of insulin and IGF. This in effect resembles a brain-restricted form of diabetes mellitus, coined "type 3 diabetes" (Steen et al., 2005). In the early stages of AD, cerebral glucose utilization is reduced by as much as 45 percent and blood-flow by around 18 percent. Cerebral metabolism seems to be declining before the onset of actual cognitive symptoms. Analysis of postmortem human brains has demonstrated that AD is associated with significantly reduced expression of insulin/IGF trophic factors and IRS (Insulin Receptor Substrate, which carries the signal from the insulin receptor to the cell core) proteins. These abnormalities increase with disease severity. Two main pathophysiological mechanisms of insulin/IGF resistance in AD have been suggested (de la Monte, 2009):

1. A progressive loss of insulin/IGF responsive neurons, following withdrawal of trophic factors.
2. An impaired insulin/IGF ligand-receptor binding due to pathological changes in membrane lipid composition, as well as a reduced expression of membrane receptors.

There seems to be links between dysfunctional insulin/IGF-signaling and neuropathological changes observed in AD. Insulin influences the metabolism of Aβ, in part by promoting its secretion and inhibiting its degradation by insulin-degrading enzyme. Therefore, impaired insulin signaling disrupts the normal processing of Aβ. At the same time, Aβ adversely affects insulin signaling by competing with and inhibiting insulin-binding, or by reducing the affinity for insulin to bind to its own receptor (Xie et al., 2002). In the healthy brain, insulin and IGF-I support neuronal cytoskeletal function via phosphorylation, which is a requisite for cytoskeleton assembly and stabilization (Hong & Lee, 1997). Impaired insulin or IGF-I signaling, as is suggested to occur in AD, can result in hyperphosphorylation of tau due to disruption of these physiological processes (de la Monte, 2009). Recent data also suggest that astrocytes produce IGF-binding protein 3 (IGFBP-3) under influence of Aβ, which in turn induces the phosphorylation of tau in neurons. This suggests that IGFBP-3 is a link between Aβ and tau pathology (Watanabe et al., 2015).

Insulin/IGF resistance also leads to increased oxidative stress, mitochondrial dysfunction, DNA damage and cell death (de la Monte, 2009).

Human and experimental studies have also suggested neurodegeneration associated with peripheral insulin resistance (as seen in diabetes mellitus type 2), likely caused by toxic lipids, including ceramides, that cross the blood-brain barrier (BBB) and trigger insulin-resistance in the brain, oxidative stress, neuroinflammation and cell death (de la Monte, 2009).

Interestingly, intranasal insulin therapy improved verbal memory recall in a test situation, for memory impaired patients with AD and MCI (who were not *APOE* ε4 carriers) (Reger et al., 2008). Metformin, a biguanide antihyperglycemic drug commonly used in diabetes mellitus type 2, provides significant neuroprotection in that the down-regulation of the insulin receptor caused by Aβ-plaques and oligomeres is significantly reduced (Chen et al., 2009). Used together, metformin and insulin might significantly improve cognitive performance and slow the rate of neurodegeneration in AD (de la Monte, 2012).

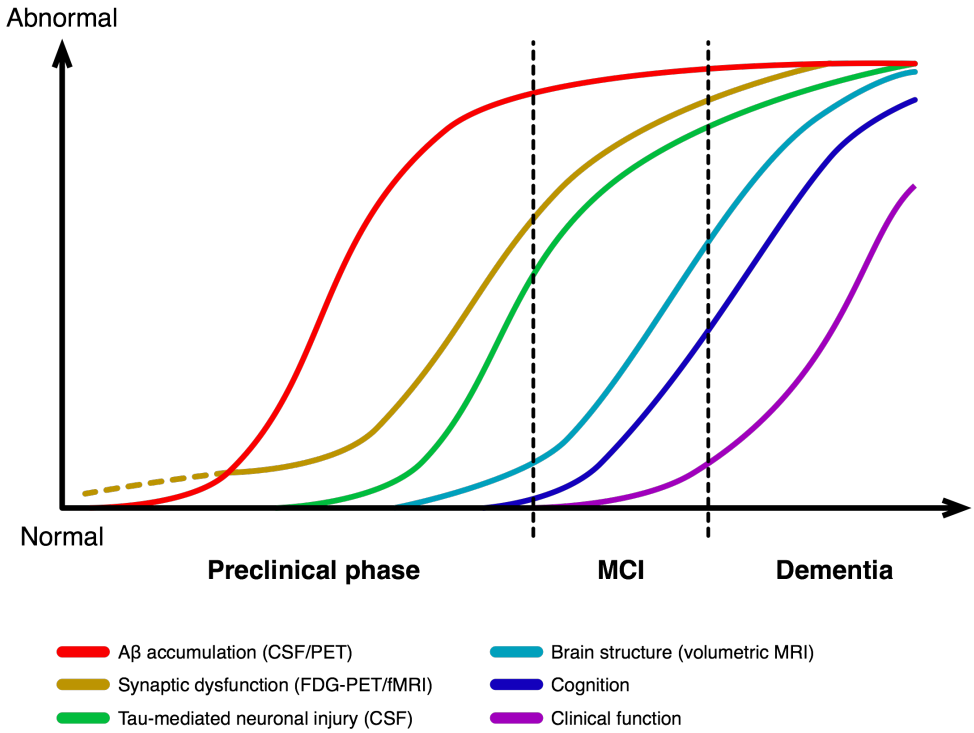
Biomarkers

Biomarkers are parameters (physiological, biochemical or anatomical) that can be measured in living organisms and that reflect specific pathophysiological processes occurring in a disease. In AD, the most widely used biomarkers can be divided into two main groups:

1. Biomarkers of Aβ-accumulation; usually abnormal tracer-retention on amyloid PET-imaging or low levels of Aβ in CSF.
2. Biomarkers of neuronal degeneration or injury; usually elevated levels of tau/P-tau in CSF, decreased uptake of fluorodeoxyglucose (FDG) on PET, in a specific topographic pattern involving temporo-parietal cortex, and typical atrophy on MRI.

Biomarkers of Aβ may be viewed as indicative of upstream events and they deviate the most from normal conditions before the onset of clinical symptoms. Biomarkers of neuronal injury and neuronal dysfunction may be viewed as indicative of downstream events and deviate the most from normal conditions later in the disease process.

In a research context, these biomarkers are used to establish the presence of typical AD-related neuropathological changes in subjects with no (or very subtle) clinical symptoms. At the MCI stage, they are used to establish the likelihood of AD being responsible for the clinical deficits. In dementia, biomarkers are used to increase (or decrease) the level of certainty that neuropathological changes typical of AD explains the major cognitive symptoms in an individual (Jack et al., 2011).



The temporal relationships between biomarkers as AD progresses from a presymptomatic state to dementia, as adapted from Jack et al. (Jack et al., 2010). Evidence of Aβ deposition is demonstrable many years before changes are visible with structural imaging or are noticeable as clinical symptoms.

A biomarker model may be superimposed on top of the model of the clinical disease stages. First, Aβ biomarkers become abnormal and a substantial Aβ load accumulates before the start of clinical symptoms. This lag between accumulation and symptoms may last for more than a decade (Buchhave et al., 2012; Sperling et al., 2011). Individual differences are probably explained by variations in brain reserves, cognitive reserves and coexisting pathologies. Biomarkers of synaptic dysfunction (FDG-PET, and functional MRI) may also demonstrate abnormalities at a very early stage, especially in *APOE* ε4 carriers. Biomarkers of neuronal injury (CSF tau and P-tau, as well as structural changes on MRI) come later in the disease (Sperling et al., 2011). It should be noted that the temporal relationship between Aβ pathology and tau pathology described above has been challenged by findings from neuropathology studies, where neurofibrillary changes appear to precede Aβ deposits (Braak & Braak, 1997).

The combination of low CSF Aβ42 (reflecting Aβ deposition) and elevated tau (reflecting the intensity of both acute neuronal damage and chronic neuronal degeneration) and P-tau (reflecting the phosphorylated state of tau and the for-

mation of tangles), is commonly referred to as the "AD signature". This combination of biomarkers has proven robust both in early identification of AD and in differentiating AD from other dementias (Blennow et al., 2015).

The CSF level of Aβ42 exhibits an inverse correlation with the load of Aβ deposits in neuropathological materials (Tapiola et al., 2009; Seppälä et al., 2012), as well as with a high retention of Pittsburg Compound B on PET (Fagan et al., 2006; 2009). Possibly, the CSF Aβ42/Aβ40 ratio more accurately reflects the amyloidogenic Aβ metabolism than Aβ42 alone (Hampel et al., 2010).

While these core AD biomarkers are already very useful in a clinical context, inter-assay and inter-laboratory variabilities are high, which limits their use for research (Blennow et al., 2015; Höglund et al., 2015).

In addition to these established biomarkers, novel markers that reflect other aspects of the AD molecular pathology may prove valuable. Neurogranin (Ng) is a postsynaptic protein that is involved in regulating synaptic signaling. Patients with AD have been shown to have significantly higher levels of Ng in CSF, when compared to healthy controls. The levels seem to be increased already at the MCI stage (Thorsell et al., 2010; Kvartsberg et al., 2014).

Visinin-like protein 1 (VLP-1), a protein expressed in neurons of the CNS, has been shown to be increased in the CSF of patients with AD. As a marker of neuronal injury, it seems to perform as a tau-independent marker of neurodegeneration (Höglund et al., 2015). The light-chain variant of neurofilament (NfL) seems to be increased in AD, as well as other dementias, such as VaD and FTD. It might be useful in investigating disease progression, possibly by looking at the ratio between the phosphorylated and non-phosphorylated forms (Höglund et al., 2015).

Diagnostic criteria

Recommendations from the National Institute on Aging and the Alzheimer's Association workgroup

In 1984 the NINCDS-ADRDA criteria for AD (McKhann et al., 1984) were established and were soon universally adopted. While they proved very useful during the next quarter-of-a-decade, advances in our understanding of AD, in our ability to capture markers of the pathophysiological processes and changes in the way we view this condition called for an update. In 2009, the National Institute on Aging (NIA) tasked four groups with revising the criteria and the results of their efforts were published in 2011 and 2012 (Jack et al., 2011; Sperling et al., 2011; Albert et al., 2011; McKhann et al., 2011; Hyman et al., 2012).

In contrast to the original NINCDS-ADRDA criteria, the new criteria acknowledges the fact that the clinical and pathological correspondence is not always consistent. They suggest making a distinction between the label used to denote the varying qualitative and quantitative expressions of the disease (AD-C) and the label of the pathophysiological process (AD-P) that is believed to underlie the syndrome. Today, it is universally believed that the syndrome of AD represents a progression through a continuum, from a preclinical asymptomatic phase, through mild cognitive impairment due to AD and finally to dementia due to AD. The new criteria reflect this view. They also incorporate biomarkers as an important part of the diagnostic puzzle.

According to the new criteria, dementia is diagnosed when there are cognitive or behavioral symptoms that interfere with activities of daily living, which represent a decline from a previous level of functioning and which is not explained by delirium or a major psychiatric disorder (in reality major depression or schizophrenia). The cognitive impairment involves a minimum of two of the following domains: impaired ability to form new memories, impaired reasoning and handling of complex tasks, impaired visuospatial abilities, impaired language functions and changes in personality or behavior. It is the significant interference with the ability to function at work or in common daily activities that delineates dementia from MCI.

The criteria propose the following classification criteria for AD dementia:

1. *Probable AD dementia.* These patients meet the general criteria for dementia outlined above. In addition, they also have an insidious onset of symptoms, a clear-cut history of worsening symptoms and cognitive symptoms in the form of memory impairment, together with language difficulties (typically of the word-finding type), visuospatial impairment or executive dysfunction. If there is a documented decline, or there is evidence of a causative genetic mutation (in *APP*, *PSEN1* or *PSEN2*), the diagnosis of probable AD can be used with an increased level of certainty. The diagnosis of probable AD should not be used when there also is a substantial cerebrovascular burden, in the presence of core features of DLB (other than dementia in itself), or in the presence of prominent features of semantic dementia or progressive non-fluent aphasia. Also, the diagnosis should not be used when there is evidence of another concurrent, active neurological disease, or non-neurological medical comorbidity, or use of medication, which might have a substantial effect on cognition.
2. *Possible AD dementia.* This diagnosis is for patients who meet the core clinical criteria for AD (1) in terms of the type of cognitive deficits, but who either has a sudden onset of symptoms, or is lack-

ing a documented progressive decline. This diagnosis is also used for patients with an etiologically mixed presentation, but who nonetheless meet the core criteria for AD.

3. *Probable AD dementia with evidence of the AD pathophysiological process.* In persons who meet the core clinical criteria for probable AD dementia (1), biomarkers may increase the certainty that the basis of the clinical dementia syndrome is indeed the AD pathophysiological process.
4. *Possible AD dementia with evidence of the AD pathophysiological process.* These patients meet the clinical criteria for non-AD dementia, but either have biomarker evidence of an AD pathophysiological process (both markers for Aβ deposition and neuronal degeneration/injury), or meet the neuropathological criteria for AD.
5. *Pathophysiologically proved AD dementia.* This diagnosis is reserved for patients who meet the clinical criteria for AD dementia (1) as well as have evidence of AD pathology in a neuropathological examination.
6. *Dementia unlikely to be due to AD.* The patient does not meet the clinical criteria for AD dementia. The diagnosis is also applicable in cases where there is sufficient evidence for an alternative diagnosis that rarely overlaps with AD, or in cases where both categories of biomarkers (Aβ deposition and neuronal injury) are negative.

MCI due to AD

The earlier criteria for MCI were slightly refined in 2011, by the workgroup tasked by NIA-AA (Albert et al., 2011). They first define core criteria for MCI as follows:

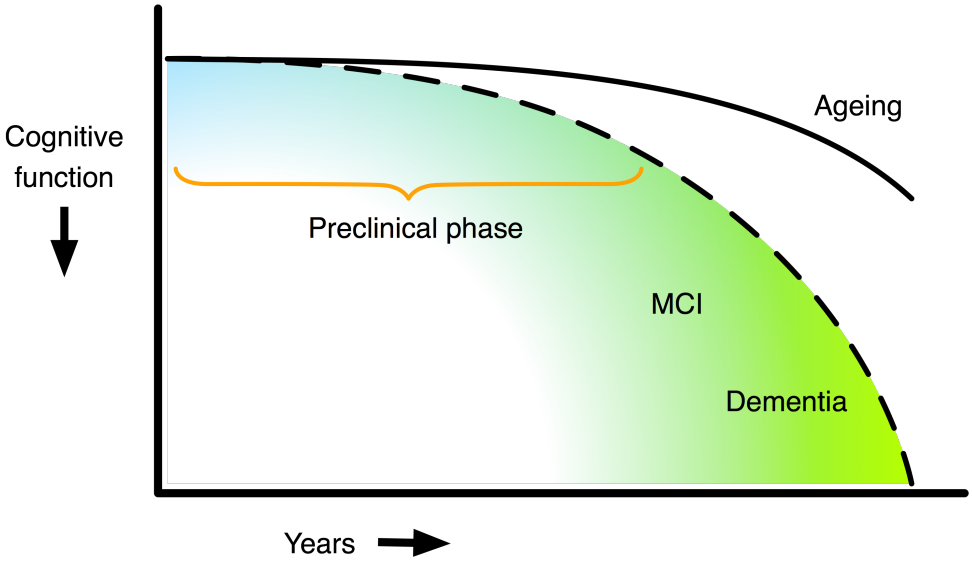
1. *Cognitive concern reflecting a change in cognition reported by the patient, informant or clinician.* The latter might be in the form of a historical or observed evidence of decline over time.
2. *Objective evidence of impairment in one or more cognitive domains.* On cognitive tests these patients usually score 1 to 1.5 standard deviations below the mean for their age– and education–matched peers in affected cognitive domains.
3. *Preservation of independence in functional abilities.* These patients may need more time, be less efficient and make more errors when performing complex tasks than in the past, but they nevertheless maintain their independence.

4. *Not demented.* These patients must not meet the general criteria for dementia.

In a clinical setting, evidence of a progressive decline in cognition and the absence of vascular, traumatic and other medical causes of cognitive decline suggests an underlying AD pathophysiological process, suggesting MCI due to AD. When present, evidence of known autosomal dominant genetic risk factors, such as mutation in the *APP*, *PSEN1* or *PSEN2* gene, also makes an underlying AD-pathology very likely. The presence of one or two *APOE* ε4 alleles is also considered to increase the risk for late-onset AD.

In a research setting, biomarkers for Aβ deposition and neuronal injury may also be used to assess the likelihood of an underlying AD pathology. Thus, patients meeting the core criteria of MCI and who also have positive biomarkers both for Aβ deposition and neuronal injury are said to have a high likelihood for MCI due to AD. Patients who meet the core criteria and only have one positive category of biomarkers (and where the other category has not been analyzed) are said to have intermediate likelihood for MCI due to AD. Patients who meet the core clinical criteria, but have negative biomarkers of both categories are said to have MCI unlikely due to AD. There are also situations where biomarkers are uninformative, such as when they are in ambiguous ranges, or conflict with one another.

Progression from healthy cognitive functioning to AD



The continuum of Alzheimer's disease. The healthy ageing process usually includes some decline of cognitive functions over the decades, most noticeable at a high age. The preclinical stage of AD starts out with a similar trajectory of cognitive decline, but increasingly deviate as the patient reached the MCI-stage. Adapted from Sperling et al. (Sperling et al., 2014).

The clinical trajectory of AD spans from an asymptomatic preclinical stage, through MCI and finally ends in a syndrome of dementia. It is likely that even the preclinical stage of AD represents a continuum from completely asymptomatic individuals with biomarker evidence suggestive of AD neuropathology, to biomarker-positive individuals already showing a subtle cognitive decline suggestive of MCI. This continuum of preclinical AD would also include individuals who carry one or more *APOE* ε4 alleles and who have positive AD biomarkers, as well as carriers of autosomal dominant mutations who are in the pre-symptomatic biomarker-positive stage of their inevitable illness (Sperling et al., 2011).

ICD-10

In clinical practice in Sweden, the diagnosis of AD is based on the International Classification of Disease-10, ICD-10 (World Health Organization, 1992). It first defines dementia as a disorder with deterioration of both memory and thinking, which is sufficient to impair personal activities of daily living. The memory impairment includes registration, storage and retrieval of new information. In this classification system the symptoms must have been present for at least six months.

Secondly, its diagnostic guidelines for Alzheimer's disease then include the presence of dementia, an insidious and slow deterioration of cognition, the absence of clinical or laboratory evidence of a systemic illness or brain disease that can induce dementia and absence of a history of sudden onset of neurological signs indicative of focal brain injury. Early-onset (before age 65) and late-onset subtypes are recognized as well as atypical and mixed types (i.e., Alzheimer's disease together with vascular dementia).

DSM-5

In the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), published in 2013, the terms dementia and amnestic disorders are replaced by the new terms mild and major neurocognitive disorder (American Psychiatric Association, 2013). However, the older term dementia is still allowed in appropriate contexts. DSM-5 provides diagnostic criteria for both mild and major neurocognitive disorder, which are then followed by criteria for different etiological subtypes. It also provides an updated list of neurocognitive domains.

Major neurocognitive disorder is defined as a significant decline in one or more cognitive domains, with clear impairment in daily living. Preferably, the decline should be verified with standardized psychological testing. For major neurocognitive disorder due to AD, the debut and progression needs to be gradual and affect at least two cognitive domains. Also either (1) evidence of genetic mutation known to cause AD or (2) affected memory functions, even progression without prolonged plateaus and no evidence of mixed pathology needs to be present.

DSM-5 defines minor neurocognitive disorder as a mild to modest decline in one or more cognitive domains, which does not interfere the capacity for independent living. This definition encompasses MCI due to AD, as defined by NIA-AA (Albert et al., 2011), but is also wide enough to include a more diverse group of entities, such as younger individuals and impairments that may be transient, static or even reversible (Sachs-Ericsson & Blazer, 2015).

Therapeutic approaches

There is no known cure for AD, but there are a few pharmacological options that mitigate the symptoms for many patients. The acetylcholine-esterase inhibitors (donepezil, rivastigmin and galantamin) all strengthen the cholinergic neurotransmitter systems by inhibiting the enzymatic breakdown of acetylcholine in the synaptic clefts, thus increasing the amount of this neurotransmitter available for surviving neurons. In Sweden, treatment with these drugs is commonly started in

mild to moderate disease. The second option is memantine, a NMDA-receptor antagonist which inhibits prolonged influx of calcium ions, and thereby protects against neurotoxicity. In Sweden, treatment is traditionally started in moderate to severe disease (although there now is a trend to start treatment already in mild dementia), and sometimes gives an additive effect to that of acetylcholine-esterase inhibitors (Wahlund et al., 2011).

For more than a decade, attempts have been made to use the human immune system to combat the changes seen in AD. Both active and passive anti-Aβ immunotherapies have demonstrated a clearance of brain Aβ deposits, but have proven to be a disappointment during Phase III clinical trials. A possible exception might be the antibody solanezumab, which has shown encouraging results in a subgroup of mildly affected AD patients. A second generation of active Aβ vaccines and new passive anti-Aβ immunotherapies seems to be under clinical testing, mostly in prodromal AD, in presymptomatic subjects with AD-related mutations and in asymptomatic subjects at risk of developing AD (Panza et al., 2014b; 2014a). Recently, Bioigen's anti-Aβ monoclonal antibody aducanumab showed reduced Aβ plaques in prodromal and mild AD in both *APOE* ε4 carriers and non-carriers, causing a stir in the scientific community (Sevigny et al., 2008).

Aims and objectives

The overreaching objective for this thesis was to explore pathophysiological processes in Alzheimer's disease, beyond mechanisms solely involving Aβ. We also wanted to explore novel biomarkers for this disease. The objectives for the individual papers were:

1. To investigate the relationship between AD pathology represented by CSF P-tau and Aβ42, the presence of *APOE* ε4 alleles and white matter lesions in patients who progress from MCI to AD.
2. To investigate how the CSF/plasma albumin ratio, as a proxy for the integrity of the blood-brain barrier, is affected in major dementias. We also wanted to study mechanisms behind a disruption of the barrier and try to identify risk factors associated with it.
3. To investigate the IGF-related system in AD, by comparing levels of IGF-I, IGF-II. IGFBP-2 and IGFBP-3 in CSF and blood plasma between cognitively healthy controls and patients with a clinical diagnosis of AD.
4. To investigate two promising biomarkers, neurogranin and YKL-40, in AD and other major dementias, and to compare their diagnostic performance to established CSF biomarkers for AD.

Material and methods

Patients and healthy controls

MCI

The patients with MCI in paper one (159 patients), paper two (96 patients and 213 patients in two different cohorts) and paper four (97 patients) all originally sought healthcare for milder memory complaints, and were diagnosed as suffering from mild cognitive impairment by clinicians experienced in the field. At the clinical baseline visit the patients underwent a clinical interview with focus on cognitive symptoms and performance in activities of daily living, as well as a thorough physical, neurological and psychiatric examination. Cognitive tests, analysis of *APOE* genotype, and CT or MRI scans of the brain were performed.

Patients with MCI at baseline met the criteria advocated by Petersen and colleagues (Petersen, 2004), including: (1) memory complaints, preferably verified by an informant; (2) objective impairment of memory functions adjusted for age and education, as judged by the clinician; (3) preservation of general cognitive functioning, as determined by the clinician's judgment based on a structured interview with the patient and a Mini-Mental Status Examination (MMSE) score greater than or equal to 24; (4) minimal impairment of daily life activities; and (5) not fulfilling the DSM-IIIR criteria for dementia (American Psychiatric Association, 1987). Patients with other causes of cognitive impairment, such as subdural hematoma, brain tumor, CNS infection, schizophrenia, major depressive episode or current alcohol abuse were not included. As is described in paper two, the patients with mild cognitive complaints in the second cohort of this study had to fulfill additional criteria and were further sub classified as subjective cognitive decline (SCD; 171 patients) or MCI (213 patients).

The MCI cohorts in the different papers overlap.

Patients with dementia

All patients with dementia were recruited from a clinical setting at the Memory Clinic in Malmö, Sweden. They all met the general criteria for dementia as defined in DSM-IIIR (American Psychiatric Association, 1987). Patients with AD (75 in paper two, 92 in paper three and 74 in paper four) met the criteria for probable AD, as defined by NINCDS-ADRDA (McKhann et al., 1984). Patients with VaD (28 in paper two and 34 in paper four) met the NINDS-AIREN criteria for VaD (Román et al., 1993). Patients with DLB/PDD (34 in paper two and 47 in paper four) met the criteria of probable DLB according to the 2005 consensus criteria (Geser et al., 2005). Patients with FTD (41 in paper two and 33 in paper four) met the 1998 consensus criteria for FTD (Neary et al., 1998).

The dementia cohorts in the different papers overlap.

Healthy controls

The cognitively healthy controls in this project were all recruited from the city of Malmö, Sweden. They were not allowed to have any cognitive complaints or any significant neurological or psychiatric illness and they needed to have a well-preserved general cognitive functioning. All controls were assessed with either a MRI scan or a CT scan of the brain, after a careful clinical interview together with at least an assessment of global function (Mini-Mental State Examination, MMSE) and tests of visuospatial and executive function (cube-drawing test and clock test), to rule out mild cognitive symptoms. As is described in paper two, the controls in the second cohort of this study were subjected to additional criteria.

The control cohorts in paper one (38 individuals), paper two (65 individuals and 294 individuals, in two different cohorts), paper three (72 individuals) and paper four (53 individuals) overlap.

Biochemical analyses

For all patients and controls, blood plasma and CSF samples were drawn at some point between 8 a.m. and 12 a.m. The procedure and analysis of the CSF followed the Alzheimer's Association Flow Chart for CSF biomarkers (Ewers et al., 2015).

For paper one and three, the levels of Aβ42, tau and tau phosphorylated at Thr181 (P-tau) were measured using Luminex xMAP technology (Luminex Corporation, Austin, Texas, USA).

For paper two, CSF levels of Aβ42, Aβ40 and Aβ38 were analyzed by Euroimmun immunoassay (EUROIMMUN AG, Lübeck, Germany.

Commercial kits from Mediagnost GmbH (Reutlingen, Germany) were used for all analyses of the IGF system in paper three. Levels of IGF-II and IGFBP-2 were analyzed using a sandwich enzyme-linked immunosorbent assay (ELISA), while radioimmunoassays (RIAs) were used for IGF-I and IGFBP-3.

For paper four, CSF neurogranin was measured using an in-house sandwich enzyme-linked immunosorbent (ELISA) assay, as described previously (Kvartsberg et al., 2014). CSF levels of YKL-40 were measured using a commercially available ELISA kit (R&D Systems, Minneapolis, MN, USA). CSF Aβ42, Aβ40 and tau were analyzed using Euroimmun (EI) (EUROIMMUN AG, Lübeck, Germany) immunoassay.

^{18}F-flutemetamol PET

For our second cohort in paper two, cerebral Aβ deposition was visualized using the PET-tracer ^{18}F-flutemetamol. PET/CT scanning of the brains was performed at two sites, using the same type of scanner (Philips Gemini). Sum images from 90 to 110 minutes after injection were analyzed using the software NeuroMarQ (GE Healthcare). A volume of interest (VOI) template was applied for the following bilateral regions: prefrontal, parietal, lateral temporal, medial temporal, sensorimotor, occipital, anterior cingulate, posterior cingulate/precuneus and global neocortical composite region. The standardized uptake value (SUVR) was defined as the uptake in a VOI, normalized for the cerebellar uptake.

Assessment of white matter lesions

All white matter lesions (WMLs) on CT and MRI scans where assessed with a protocol developed by Wahlund et al. (ARWMC, Age Related White Matter Changes scale) (Wahlund et al., 2001). This scale has been developed for rating both MRI and CT images, with a high level of agreement between the modalities.

The WMLs were assessed separately in the left and right frontal regions, the basal ganglia and the occipital-parietal regions (in paper one simply referred to as the parietal region since most WMLs were located here). Temporal lesions were not rated since this is a very rare location for WMLs and therefore unsuitable for statistical analysis (Bronge, 2002).

Statistical analyses

In paper one, a non-parametric Kruskal-Wallis test followed by Mann-Whitney U-test for continuous variables was used to compare demographic and CSF baseline data between groups. Pearson´s $\chi 2$ test was used for dichotomous variables and the Spearman correlation coefficient was calculated in bivariate correlation analyses. To compare the extent of WMLs between individuals with pathological and non-pathological CSF levels of P-tau, as well as between individuals with and without at least one *APOE* ε4 allele, a Mann-Whitney test was performed for each brain region. A Kaplan-Meier estimate, together with a Cox-Regression, adjusted for age and gender, was used for analyzing the probability of conversion from MCI to AD.

In paper two, we used univariate general linear models (ANCOVA) for group-wise comparisons. Linear regressions were used to explore associations between two continuous variables. Because data for some of the analyses were skewed, all variables were ln-transformed before analyses. By using Pearson's correlation analysis and Student's T-tests, we found the majority of the analytes to be correlated with age and gender differences. Because of this all statistical analyses were controlled for age and gender. The study participants were categorized into groups with normal and pathological PET status using the SUVR cutoff>1.42. Associations between the CSF/plasma albumin ratio and ^{18}F-flutemetamol SUVR as well as vascular risk factors were tested in the total sample with univariate and linear regression models, controlling for age, gender and diagnosis.

In paper three, Mann–Whitney nonparametric U tests were used for comparing age and MMSE scores between the two groups, while a Pearson's $\chi 2$ test was used for comparing gender distribution and vascular risk factors. In order to adjust for the potentially confounding effects of age, continuous variables were log-transformed, before a univariate general linear model (ANCOVA) was used for each biomarker, with age included as a covariate in the analyses. We then performed ANCOVA analyses for each biomarker, with both age and body mass index (BMI) included as covariates. Even though the gender distribution did not differ in a statistically significant way between the two groups, we also performed an ANCOVA analyses for each biomarker, with age, BMI and gender included as covariates. Because of the high coefficients of variation (CV) for the analyses of IGF-I, we also excluded cases with a CV >20% in a separate analysis. Spearman's correlation coefficient was calculated in a bivariate correlation analyses.

In paper four, neurogranin and YKL-40 levels did not present a normal distribution and were therefore ln-transformed before analysis. There were significant differences in age (as tested by a one-way analysis of variance, ANOVA) and gender (tested using Pearson's $\chi 2$ test) between the diagnostic groups. Therefore, for group-wise comparisons of neurogranin and YKL-40, we used univariate general linear models (ANCOVA) controlling for age and gender. Cognitively stable

MCI patients (sMCI), MCI patients who later progressed to AD (prodromal AD/MCI-AD) and AD dementia patient were included in all analyses as separate diagnostic categories. The diagnostic accuracy of CSF biomarkers was assessed with a receiving operating characteristic (ROC) curve analysis. Differences in the area under the curve (AUC) of two ROC curves were compared using bootstrap method (Robin et al., 2011).

Ethical considerations

The Regional Ethics Committee in Lund, Sweden, approved this project and the patients and/or their relatives, gave their informed consent for research. All study procedures was conducted in accordance with the Helsinki Declaration.

Main results

The main features of the four papers are presented below, while the papers in their entirety are found in the appendix.

Paper one

Tau pathology and parietal white matter lesions have independent but synergistic effects on early development of Alzheimer's disease

WMLs are common in older populations and there are earlier contradictory findings suggesting them to increase the risk of future dementia in patients with MCI. While previous studies have investigated the role of WMLs in fully developed dementia due to AD, we wanted to investigate how these changes affect patients with less severe amnestic symptoms.

In this study we investigated the relationship between AD pathology, represented by CSF P-tau and Aβ42, the presence of *APOE* ε4 alleles and the presence of WMLs in patients who progress from MCI to AD (prodromal AD). We did this by comparing levels of tau, P-tau and Aβ42 in CSF, the presence of WMLs in the brain, and the *APOE* genotype in 159 patients with MCI and 38 cognitively healthy controls.

Results

Patients with prodromal AD showed significantly more WMLs in the parietal lobes at baseline, when compared to healthy controls or those with stable MCI. In the prodromal AD group, the vast majority (90%) showed pathological CSF levels of Aβ42, but only a subset had pathological levels of P-tau (46%) or WMLs visible on brain scans (45%). Those with normal levels of CSF P-tau had more WMLs in the parietal regions than those with pathological P-tau levels. Also, those without an *APOE* ε4 allele had more WMLs in the parietal lobes than those with at least one allele. Further, MCI patients with both pathological P-tau levels and pa-

rietal WMLs showed a greater risk of developing AD than those with just one of the two pathological parameters.

Conclusion and comments

In this retrospective cohort study, patients who sought healthcare with milder memory complaints showed less tau pathology when they also exhibited WMLs in the parietal lobes.

Evidence of Aβ deposition (such as lower CSF Aβ levels) and of neuronal degeneration (such as elevated tau and P-tau) usually precede the onset of cognitive symptoms in AD (Sperling et al., 2011). In our material, almost all the patients with prodromal AD had low levels of CSF Aβ42, while less than half had high CSF P-tau. This implies that the patients with normal P-tau needed the addition of other factors to develop the cognitive symptoms that prompted them so seek healthcare. We suggest that WMLs in the parietal lobes might be such a factor, which in its own right contributes to a clinical presentation of AD.

We also found those with both pathologically elevated levels of P-tau and presence of WMLs in the parietal lobes to progress more rapidly towards dementia, when compared to those with only one of these two markers. We hypothesize that the added effect of WMLs in the parietal regions amplify the impact of AD pathology and thereby lower the threshold for symptoms to emerge.

The reserve capacity of the brain can be viewed as the sum of a lifetime's worth of protecting and detrimental factors. This reserve is thought to buffer the effects of different pathologies affecting the brain and it might account for the often reported discrepancy between the degree of observed brain damage and its clinical manifestations (Stern, 2009; 2002). Based on the findings in our study, we suggest that WMLs in the parietal lobes erode the reserve capacity of the brain, thereby making it harder to compensate for core AD pathology.

As we discuss in our paper, earlier work has shown that vascular pathology is a common feature in AD (Englund, 1998; Schneider et al., 2007). The addition of vascular lesions to AD pathology also seems to worsen the clinical symptoms of disease in already demented individuals (Esiri et al., 1999; Heyman et al., 1998; Snowdon et al., 1997). A few of theses studies have observed less extensive tau pathology in demented patients with vascular lesions, which in part matches our findings.

We attempted to study the relationship between WMLs and AD pathology already at the MCI stage and to see how these two pathologies affect disease progression. A more recent study found confluent WMLs to increase the risk of conversion from subjective cognitive impairment to dementia due to AD (Garcia-Ptacek et al., 2014), supporting our results.

We used a protocol developed by Wahlund et al. (Age-Related White Matter Changes Scale, ARWMC) for assessment of WMLs. This scale was developed for rating both MRI and CT images, with a high level of agreement between the modalities (Wahlund et al., 2001). While other methods for rating WML load is in use (for instance grading according to the Fazekas visual rating scale, manual volumetric methods and FreeSurfer volumetry on MRI images), we felt the ARWMC protocol closely matches the routine in a clinical setting.

In our study, patients with a diagnosis of MCI at the time of lumbar puncture and who later developed AD were considered suffering from prodromal AD. An addition of vascular disease was allowed, as long as the patient fulfilled the clinical criteria for AD. We think this accurately reflects the reality at a memory clinic, which adds to the clinical relevance of our findings.

Paper two

Increased blood-brain barrier permeability is associated with dementia and diabetes, but not amyloid pathology or APOE genotype

Proper neuronal functioning depends on a tightly regulated extracellular environment. The blood-brain barrier (BBB), a selective diffusion barrier at the level of the cerebral microvascular endothelium, dynamically regulates ion balance, facilitates nutritional transport and acts as a barrier to potentially neurotoxic molecules between the peripheral blood stream and the CNS. Disruption of this barrier has been described in various dementias, but the mechanisms behind this needs to be explored further.

In this study we used two large cohorts (one with 65 healthy controls and 274 patients with MCI or dementia; one with 292 healthy controls, 171 individuals with subjective cognitive decline [SCD] and 213 individuals with MCI) to investigate disruption of the blood-brain barrier in different dementias, using the CSF/plasma ratio of albumin as a proxy. We studied how this disruption might relate to the amyloid pathology observed in AD and to the *APOE* ε4 genotype. We also analyzed biomarkers of angiogenesis and endothelial damage in different dementias, as well as possible risk factors for disruption of the blood-brain barrier.

Results

In our material, the CSF/plasma albumin ratio was significantly elevated in AD, DLB/PDD, VaD and FTD when compared to controls, suggesting an increased

permeability of the BBB in these conditions. This elevation was not seen in patients with MCI who later developed AD.

When we compared controls, patients with SCD and patients with MCI, who all had a pathological CSF signature for Aβ (Aβ42/Aβ40<0.1), to controls with normal CSF status (Aβ42/Aβ40≥0.1), we found no differences in the CSF/plasma albumin ratio between these groups. We also analyzed these groups for differences in cortical Aβ deposition as measured by ^{18}F-flutemetamol PET and found no correlation between the CSF/plasma albumin ratio and composite ^{18}F-flutemetamol SUVR. Also, there were no differences in the ratio between study subjects with normal and pathological amyloid PET.

We did not find any differences in CSF/plasma albumin ratio between *APOE* ε4 carriers and non-carriers in any of the diagnostic groups in either of the two cohorts.

In our cohort with dementia patients, we found an association between an elevated CSF/plasma albumin ratio and increased CSF-levels of ICAM-1 and VCAM-1 in all diagnostic groups, suggesting a link between a disrupted BBB and endothelial pathology. We also found associations between an elevated CSF/plasma ratio and VEGF (or the VEGF/sVEGFR1 ratio) in all diagnostic groups except VaD, which suggests a link between disrupted BBB and angiogenesis as well. Compared to controls, CSF levels of VEGF (or VEGF/sVEGFR1) were increased in all diagnostic groups, while no differences in CSF levels of ICAM-1 or VCAM-1 were observed.

In both out cohorts we found evidence of an increased CSF/plasma albumin ratio in patients with diabetes compared to those without the disease. We also observed higher CSF-concentrations of ICAM-1, VCAM-1 and VEGF in diabetics. For a subset of our second cohort we also had access to data collected about twenty years prior to collection of the biomaterial used in this study. In this subset of individuals, BMI and waist-hip ratio at midlife predicted an increase in CSF/plasma albumin ratio 20 years later.

Conclusion and comments

We found evidence of a compromised BBB in several different dementias. This dysfunction does not seem to be directly associated with Aβ pathology or the *APOE* ε4 genotype. Instead our data link dysfunction of the BBB with abnormal angiogenic pathways, endothelial damage and diabetes mellitus.

While an elevated CSF/plasma albumin ratio is a uncontroversial finding in vascular dementia (Wallin et al., 1990; Wada, 1998; Taheri et al., 2011; Skoog et al., 1998), the accumulated evidence in AD is contradictory (Erickson & Banks, 2013).

Our data suggests a dysfunction of the BBB to be common across different dementias. Since the ratio does not seem to be elevated in cognitively healthy individuals with markers of cortical deposition of Aβ, we believe a causative link between amyloid pathology and dysfunction of the BBB to be unlikely in the early stages of AD.

In contrast to an earlier study (Halliday et al., 2013), we did not find differences in the CSF/plasma albumin ratio between *APOE* ε4 carriers and non-carriers, indicating that no causative link between dysfunction of the BBB and the *APOE* ε4 genotype exists.

Our data indicates an increased bioavailability, and consequently increased signaling, of VEGF across different forms of dementias. Interestingly, patients with MCI or prodromal AD had increased levels of VEGF in spite of a normal CSF/plasma albumin ratio, which suggests that an abnormal production of VEGF precedes an increased permeability of the BBB.

We observed associations between disruption of the BBB and diabetes in both our cohorts, in accordance with earlier findings (Starr et al., 2003; Hawkins et al., 2007). In these patients with diabetes, we also found increased levels of ICAM-1 and VCAM-1 in CSF, as well as positive correlations between the CSF/plasma albumin ratio and CSF-levels of these adhesion molecules. To the best of our knowledge, this has not previously been described. We believe this suggests that diabetes may lead to endothelial damage in the cerebral vasculature, which in turn contributes to the dysfunction of the BBB.

In our first cohort, diabetes was more prevalent among patients with dementia (17 out of 178) than controls, MCI and prodromal AD (7 out of 161). Since we found an elevated CSF/plasma albumin ratio in patients with dementia, we cannot rule out the possibility of the elevated albumin ratio seen in diabetes to be explained by this larger proportion of dementia patients in this group. Our findings are however corroborated by findings from our second cohort (controls, SCD and MCI), where no differences in CSF/plasma albumin ratios between groups was found, as well as previous studies (Hawkins et al., 2007; Starr et al., 2003).

This study was mainly designed as a cross-sectional study, analyzing data collected at a single point in time. However, for a subset of individuals we had access to life-style data collected around twenty years prior, which meant we could study possible risk factors for BBB disruption in a longitudinal manner in this smaller material.

We used the CSF/plasma albumin ratio as a proxy for the permeability of the BBB, which is considered common practice (Nägga et al., 2014; Reiber, 1994; Tibbling et al., 1977). However, this assumption is not unproblematic, since factors other than actual disruption of the barrier might influence measured levels of albumin in the CSF. In particular, the turnover rate of CSF is slowed with age and even more so in patients with AD, which results in higher CSF-levels of albumin (Erickson & Banks, 2013; Reiber & Peter, 2001). While we did adjust for the po-

tentially confounding effects of age in our material, we cannot entirely rule out the risk of other factors influencing measured ratios. In future work, direct assessment of blood-brain function, for instance by using dynamic contrast-enhanced MRI and labeled tracers, is warranted in order to confirm our findings.

Paper three

Changes in cerebrospinal fluid and blood plasma levels of IGF-II and its binding proteins in Alzheimer's disease: an observational study

The IGF-related system is implicated in neuroregeneration and cell repair, as well as regulating lifespan. The two ligands, IGF-I and IGF-II, are both potent growth-promoting and neuroprotective factors in the human nervous system. IGF-II has also been found to affect memory functions in a rat model.

In this study, which we designed as a pilot study, we explored changes in the IGF-related system in patients with AD. We did this by comparing blood plasma and CSF levels of IGF-I, IGF-II, IGFBP-2 and IGFBP-3 between 72 healthy controls and 92 patients with AD.

Results

We found significantly lower blood plasma levels of IGF-II and IGFBP-3, but higher levels of IGFBP-2 in patients with AD, compared to controls. The levels of IGF-II and IGFBP-2 were significantly elevated in the CSF from patients with AD. When we adjusted for age, body-mass index and gender, only differences in IGF-II levels and IGFBP-3 levels in blood plasma reached a statistical significance level of $p<0.05$. We also found correlations between established CSF biomarkers for AD (tau and P-tau) and components of the IGF system.

Conclusion and comments

We found differences in CSF and blood plasma levels of IGF-II and some of it's binding proteins between patients with AD and healthy controls. These differences might reflect mechanisms that are part of the pathophysiological processes in AD, or they may be part of a more general response to brain damage.

At the time of publication for this study, we were only able to find one other paper reporting on IGF-II in AD (Nordberg et al., 1993). The findings from that study in part matched our own. Recently, however, new data have emerged. Like

us, Åberg et al. found increased CSF levels of IGF-II and IGFBP-2 in patients with AD, but only in males (Åberg et al., 2015).

As we discuss in our paper, IGF-I has been more extensively studied in the context of AD, but the accumulated body of knowledge is contradictory.

In our initial analysis, we adjusted for the potentially confounding effect of age, since our patients with AD were significantly older than our controls. Besides age, also body composition and possibly gender are known to affect circulating levels of IGF-I (Duron et al., 2012). Because of this we reanalyzed our material, adjusting for the potentially confounding effects age, gender and BMI. In this analysis, everything but differences in IGF-II and IGFBP-3 in blood plasma failed to reach a statistical significance. Since we only had BMI data on a subset of individuals (88 patients and 47 controls), we believe this to be caused by a loss of statistical power, due to small sample size.

In our paper we discuss the possibility of an ineffective IGF-system that fails to uphold the neuroregenerative and neuroprotective mechanisms of a healthy brain. However, a more likely explanation for our findings would be that the observed changes in the IGF-system reflects a more general response to brain damage. The fact that a brief induction of IGF-I expression seems to follow traumatic brain injury in a mouse model supports this (Madathil et al., 2010), as does the finding that increasing the levels of circulating IGF-I improves disabilities after traumatic brain damage in humans (Devesa et al., 2013). It would be interesting to analyze the IGF-system components in other cognitive disorders as well, both neurodegenerative and vascular, to shed light on this.

The design of the study (a case control study) makes it impossible to infer any causality. Also, since the studied cohort was small, this probably led to a loss of statistical power during analyses and the risk of missing less pronounced effects. Another limitation with this study is that the *APOE* ε4 allele, which is a known risk factor for AD, was not analyzed for the controls in this material. It would be theoretically possible that the *APOE* genotype would affect the IGF-system, but we have been unable to find any literature on the subject. A later analysis of our material did not find differences in levels of IGF-II in CSF or blood plasma, IGFBP-2 in CSF or IGFBP-3 in blood plasma between *APOE* ε4 carriers and non-carriers. This makes the different frequencies of *APOE* ε4 carriers between cases and controls an unlikely explanation for our findings.

Paper four

Cerebrospinal fluid neurogranin and YKL-40 as biomarkers of Alzheimer's disease

These are several known pathophysiological processes seen in AD, besides changes in Aβ homeostasis. Biomarkers useful for early diagnosis and for monitoring other aspects than Aβ are needed, both for patient care and the development of drugs combating this disorder. In this study, we analyzed differences in CSF-levels of neurogranin and YKL-40, promising biomarkers of synaptic degeneration and neuroinflammation respectively, in a cohort of 338 individuals with cognitively healthy controls and patients with stable MCI (sMCI), prodromal AD, AD, PDD, DLB, VaD and FTD. The diagnostic accuracy of neurogranin and YKL-40 were compared with that of established AD biomarkers; Aβ42, Aβ40 and tau.

Results

We found CSF-levels of neurogranin to be higher in AD and lower in dementia of other etiologies, when compared to healthy controls. Patients with AD showed considerably increased CSF-levels of neurogranin compared to patients with DLB/PDD, VaD or FTD. CSF-levels of YKL-40 was increased in AD and FTD, when compared to healthy controls. CSF-levels of YKL-40 were also increased in AD compared to DLB/PDD, but not to VaD or FTD. Neither levels of neurogranin, nor levels of YKL-40 differed significantly between sMCI patients and prodromal AD patients. Both neurogranin and YKL-40 showed a positive correlation with CSF Aβ40 and tau. They could also separate AD dementia from non-AD dementias in a ROC-analysis of diagnostic accuracy, but neither performed better than combinations of Aβ and tau. We got similar results when we separated sMCI cases from prodromal AD cases using the same method.

Conclusion and comments

The CSF-level of neurogranin seems to be selectively increased in AD dementia, whereas YKL-40 is increased in both AD and FTD. Although, these two biomarkers do not seem to improve the diagnostic accuracy of either prodromal AD or AD dementia, when compared to core AD biomarkers in CSF, they reflect two processes—synaptic degeneration and microglial activation—that may be important in this neurodegenerative disease.

Our findings that CSF-levels of neurogranin and YKL-40 levels are increased in patients with AD is in agreement with existing data (Olsson et al., 2012; Perrin et al., 2011; Thorsell et al., 2010). Our material included patients from different stages of the AD disease process (prodromal AD as well as dementia due to AD), which made it possible to crudely study the timing of biomarker change in this disease. However, we did not find evidence of neurogranin levels to be increased in prodromal AD, which is in contrast to earlier work (Portelius et al., 2015; Kvartsberg et al., 2014). One possible explanation for this is that we had too few patients in the prodromal AD group to be able to detect smaller effects.

Interestingly, we found patients with FTD and VaD to have significantly lower levels of neurogranin, when compared to healthy controls. This is something we hope will be further investigated in future work.

This study was in part designed as a case-control study, which enabled us to find associations between disease and biomarkers, but not infer any causality. In part, it was also designed as a cross-sectional study, comparing the diagnostic performance of these two biomarkers with established CSF biomarkers for AD.

It is possible that neurogranin can be used for monitoring the effects of future disease-modifying therapies on synaptic integrity, while YKL-40 might be used to investigate the effects of novel drugs affecting neuroinflammation. Before that can happen, further study of these biomarkers is needed, preferably in the form of longitudinal studies, with CSF sampling at different time-points during the course of disease.

Concluding discussion

The amyloid cascade hypothesis posits that the aggregation of Aβ triggers a complex neurodegenerative cascade, ultimately leading to dementia due to AD (Golde et al., 2011). Even though a lot of experimental and observational data supports it, this theory has thus far not been particularly useful when it comes to developing new treatments for AD. Even if this hypothesis would be true, it does not necessarily mean that the accumulation of Aβ is the best target for therapeutic intervention.

If the accumulation of Aβ indeed acts as the spark that kindles the disease process, it would be rational to deploy anti-Aβ therapies before the accumulation has started (prevention), or at the very least early in the disease (secondary prevention). In contrast, the therapeutic studies to date have been performed on patients further along the AD spectrum (Golde et al., 2011; Karran et al., 2011). Apart from one small glimmer of hope, they have all proved disappointing (Toyn, 2015).

Since proper testing of the preventive properties of anti-Aβ candidate drugs would in all likelihood require a timeline of 15 to 20 years (Golde et al., 2011), it would be wise to in parallel investigate other pathophysiological mechanisms in AD, where possible targets for therapy may be found. Even if anti-Aβ therapies ultimately proves a success, there will still be a need for treatments effective for all those patients who did not have access to prevention and thus have progressed towards dementia. The overreaching aim of this thesis was to explore such pathophysiological mechanisms, which might contribute to the development of dementia due to AD, or which might improve possibilities for an early diagnosis in this disease.

In the work presented in this thesis we suggest that WMLs in the parietal lobes and tau pathology both have independent effects on the reduction of the cognitive reserve capacity of the brain. MCI-patients who had evidence of both tau pathology in CSF and WMLs visible on brain scans had a much higher risk of progressing to dementia due to AD.

We also saw that the permeability of the BBB is increased in major dementia disorders, but that it does not seem to be related to Aβ pathology or the *APOE* genotype. Instead, it seems to be associated with diabetes and damage to the microvasculature of the brain.

We found that CSF and blood plasma levels of IGF-II and some of its binding proteins are changed in patients with AD, in particular IGF-II and IGFBP-2 in CSF.

Lastly, we tested the usefulness of neurogranin and YKL-40, two promising biomarkers for two different pathophysiological processes in AD (synaptic degeneration and microglial activation). We found that they did not improve the diagnostic accuracy or either prodromal AD or AD dementia, when compared with core CSF AD biomarkers.

The studies in this thesis were performed using clinically relevant (and in most cases also large) materials, which makes it easier to generalize our findings to the real-world scenario at a memory clinic. An obvious shortcoming is that the clinical diagnoses of our patients were not verified by neuropathological assessment in most cases. Such an assessment is for the most part not performed in the clinical setting from which our patients were recruited. Also, an effort to have all our patients examined by a neuropathologist would have resulted in very long follow-up periods, which was beyond the scope of this thesis. Instead, all patients were examined by medical doctors with both interest and aptitude for cognitive disorders, which we think adds to the usefulness of the resulting knowledge in everyday healthcare.

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