Biomarkers for Alzheimer's disease and the *APOE* polymorphism

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Abstract

Alzheimer's disease (AD) is the most common form of dementia and cerebrospinal fluid (CSF) biomarkers reflecting the core pathology of AD are now widely used for diagnosis making, in particular β-amyloid$_{1-42}$ (Aβ42) reflecting amyloid plaque pathology, phosphorylated tau (P-tau) reflecting neurofibrillary tangle pathology and total tau (T-tau) reflecting general neurodegeneration. In addition, blood-based biomarkers for AD are in the pipeline with recent studies showing promising diagnostic potential. The most important genetic risk factor for sporadic AD is the ε4 allele of the apolipoprotein E (*APOE*) polymorphism, increasing risk for AD diagnosis in a dose-dependent manner as well as lowering the age of onset.

We conducted a comprehensive meta-analysis of the AD biomarker literature from 1984 to 2014, which could confirm the robust diagnostic performance of the above-mentioned established CSF biomarker triad for AD, and also revealed possible new biomarker candidates in both CSF and blood that could contribute to the diagnostic work-up of the disease as well as serve as tools for monitoring new disease-modifying treatments. In a large multicentre study, we confirmed the strong association between the *APOE* ε4 genotype and AD and showed that the ε4 allele also affects concentrations of CSF Aβ42 in a dose-dependent manner. However, the *APOE* polymorphism does not blur the diagnostic accuracy of the established AD biomarkers and CSF Aβ42 was shown to reflect cerebral amyloid pathology irrespective of the *APOE* genotype. In another multicentre cohort consisting of solely cognitively healthy subjects, we showed that the dose-dependent effect of *APOE* ε4 on CSF Aβ42 was absent in younger subjects and CSF Aβ42 concentrations started to drop around age 50 and even earlier in ε4-carriers, pinpointing the earliest disturbances in amyloid homeostasis, long before cognitive impairment becomes apparent.

Taken together, the results from this thesis underline the usefulness of AD biomarkers as well as their robust diagnostic performance irrespective of the most prominent genetic risk factor. In addition, since biomarkers (in particular CSF Aβ42) can reflect pathological changes already in the preclinical stage of the disease, they could become valuable in future AD prevention, once disease-modifying therapies become available.

Populärvetenskaplig sammanfattning

Alzheimers sjukdom (AD) är den vanligaste demensformen och diagnosen ställs idag bland annat med hjälp av så kallade biomarkörer, dvs. biologiska ämnen som kan mätas i olika kroppsvätskor och som återspeglar sjukliga processer i kroppen. Ett kännetecken vid AD är utfällningar (plack) i hjärnan som består av proteinet β-amyloid$_{1-42}$ (Aβ42). Aβ42 kan mätas i ryggmärgsvätska (cerebrospinalvätska, Csv) och halten är vanligen sänkt vid AD. Dessutom kan man mäta proteinet tau (T-tau) som läcker ut från sönderfallande nervceller och halten i Csv är därför hög vid AD. En strukturellt förändrad variant av tau (fosforylerat tau, P-tau) är typisk vid AD och även denna kan mätas i Csv, där höga halter är relativt specifika för just AD. Det är känt att risken att insjukna i AD inte är slumpmässig utan åtminstone delvis ärftligt betingad. En så kallad sårbarhetsgen som varit känd sedan länge är *APOE*, som föreligger i tre olika varianter, varav en (*APOE* ε4) är förknippad med en ökad risk att insjukna i AD.

Inom ramen för denna avhandling genomfördes en stor granskning av hela biomarkörlitteraturen för AD från 1980-talet tills nu, där vi kunde bekräfta att de etablerade biomarkörerna är mycket robusta och träffsäkra. Dessutom uppdagades nya lovande biomarkörer som skulle kunna användas i diagnostiken framöver. I en annan stor studie med Alzheimerpatienter från olika centra både i Sverige och utomlands kunde vi se att riskgenen *APOE* kan påverka halten av biomarkörer (i synnerhet Aβ42) i Csv, där bärare av *APOE* ε4-varianten har lägre halter jämfört med icke-bärare. Trots detta är de biomarkörer som används idag mycket träffsäkra oavsett vilken *APOE*-uppsättning som föreligger. I en annan studie undersökte vi ett stort antal friska individer i ett brett åldersintervall och kunde visa att *APOE* ε4-varianten inte påverkade halten av Aβ42 i Csv bland de allra yngsta. Däremot börjar nivåerna sjunka redan från 50-års åldern, och ännu tidigare bland bärare av *APOE* ε4 varianten, vilket kan indikera att det försiggår sjukliga processer i hjärnan långt innan några minnesstörningar blir märkbara för patienten. Resultaten från denna avhandling understryker att biomarkörer är användbara inte bara för att ställa en Alzheimerdiagnos utan också för att hitta tidiga förändringar innan patienten blir sjuk, vilket kan bli värdefullt framöver ifall en förebyggande behandling mot AD kan bli verklighet.

Populärwissenschaftliche Zusammenfassung

Die Alzheimer-Krankheit, auch Alzheimer-Demenz (AD) genannt, ist die häufigste Form der Demenzerkrankungen und die Diagnose wird heutzutage unter anderem mit Hilfe sogenannter Biomarker gestellt. Biomarker sind in verschiedenen Körperflüssigkeiten meßbare biologische Stoffe, die krankhafte Prozesse im Körper widerspiegeln. Ein charakteristisches Kennzeichen der Alzheimererkrankung sind extrazelluläre Fällungen im Gehirn, welche als Hauptbestandteil das Protein β-amyloid$_{1-42}$ (Aβ42) enthalten. Aβ42 kann in der Gehirn-Rückenmarksflüssigkeit (dem Liquor cerebrospinalis) gemessen werden und die Konzentration bei der AD ist üblicherweise erniedrigt. Desweiteren ist das Protein Tau (T-tau), welches von zerfallenden Nervenzellen freigesetzt wird, im Liquor meßbar und bei der AD liegen oft erhöhte Konzentrationen von T-tau vor. Eine durch eine Vielzahl an Phosphorylierungen strukturell veränderte Form des Tau Proteins (P-tau) ist typisch für die AD und auch hier können erhöhte Konzentrationen im Liquor gemessen werden. Es ist bekannt, daß das Risiko, an der sporadischen Form der AD zu erkranken, einem erblichen Faktor unterliegt, nämlich dem *APOE* Gen. Jenes Gen liegt in drei Varianten vor, von denen eine (*APOE* ε4) mit einem erhöhten Erkrankungsrisiko vergesellschaftet ist.
Im Rahmen dieser Dissertation wurde eine umfassende Durchsicht der gesamten Literatur über Biomarker der AD durchgeführt, welche sich von den 80er Jahren bis in die Gegenwart erstreckt. Dabei konnten wir einerseits bestätigen, daß die oben genannten etablierten Biomarker äußerst robust sind und eine hohe Treffsicherheit aufweisen. Andererseits traten auch neue Biomarker als vielversprechende Kandidaten hervor, welche möglicherweise zukünftig in das diagnostische Arsenal aufgenommen werden könnten. In einer weiteren umfassenden Studie mit Alzheimerpatienten von verschiedenen Zentren, sowohl aus Schweden als auch aus anderen Ländern, konnten wir feststellen, daß das Risikogen *APOE* die Konzentrationen von Biomarkern im Liquor (insbesondere Aβ42) beeinflussen kann, wobei Träger von *APOE* ε4 niedrigere Liquorkonzentrationen aufweisen als jene mit anderen *APOE* Genvarianten. Dieses Sachverhaltes zum Trotz ist Aβ42, sowie auch die anderen oben genannten Liquorbiomarker, überaus treffsicher, ungeachtet der genetischen Zusammensetzung des *APOE* Genes. In einer

anderen Studie mit ausschließlich gesunden Kontrollpersonen in allen Altersgruppen konnten wir zeigen, daß die Konzentration von Aβ42 im Liquor der jüngsten Studienteilnehmer nicht vom *APOE* Genotyp beeinflußt wird. Allerdings konnten mit steigendem Alter sinkende Konzentrationen von Aβ42 festgestellt werden, wobei dieser Rückgang bereits ab dem Alter von 50 Jahren zu beobachten war, und sogar noch früher bei Trägern der *APOE* ε4 Genvariante. Dieses Phänomen deutet darauf hin, daß krankhafte Prozesse im Gehirn stattfinden können, lange bevor ein eventueller Gedächtnisverlust für den Patienten bemerkbar wird.

Die Ergebnisse dieser Doktorarbeit unterstreichen, daß Biomarker nicht nur für die Diagnostik der Alzheimererkrankung von Bedeutung sind, sondern auch dazu verwendet werden können, um Zeichen früher krankhafter Veränderungen aufzuzeigen, welche bereits vor dem eigentlichen Ausbruch der Erkrankung vorliegen. Dieser Anwendungsbereich könnte enorm an Bedeutung gewinnen, sollte es möglich werden, in Zukunft der Erkrankung mit neuen wirksamen Arzneimitteln vorbeugend entgegenzutreten.

List of papers

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Olsson B, **Lautner R**, Andreasson U, Öhrfelt A, Portelius E, Bjerke M, Hölttä M, Rosén C, Olsson C, Strobel G, Wu E, Dakin K, Petzold M, Blennow K and Zetterberg H.

CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis.

Lancet Neurology. 2016; **15**(7): p. 673–684.

II. Andreasson U, **Lautner R**, Schott JM, Mattsson N, Hansson O, Herukka SK, Helisalmi S, Ewers M, Hampel H, Wallin A, Minthon L, Hardy J, Blennow K and Zetterberg H.

CSF biomarkers for Alzheimer's pathology and the effect size of APOE ε4.

Molecular Psychiatry. 2014; **19**(2): p. 148–149.

III. **Lautner R**, Palmqvist S, Mattsson N, Andreasson U, Wallin A, Pålsson E, Jakobsson J, Herukka SK, Owenius R, Olsson B, Hampel H, Rujescu D, Ewers M, Landén M, Minthon L, Blennow K, Zetterberg H, Hansson O and the Alzheimer's Disease Neuroimaging Initiative.

Apolipoprotein E genotype and the diagnostic accuracy of cerebrospinal fluid biomarkers for Alzheimer disease.

JAMA Psychiatry. 2014; **71**(10): p. 1183–1191.

IV. **Lautner R**, Insel PS, Skillbäck T, Olsson B, Landén M, Frisoni GB, Herukka SK, Hampel H, Wallin A, Minthon L, Hansson O, Blennow K, Mattsson N and Zetterberg H.

Preclinical effects of APOE ε4 on cerebrospinal fluid Aβ42 concentrations.

Alzheimer's Research & Therapy. 2017; **9**(1): p. 87.

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Abbreviations

Aβ	β-amyloid
Aβ42	β-amyloid$_{1-42}$
AChE	Acetylcholinesterase
AD	Alzheimer's disease
ADNI	Alzheimer's disease Neuroimaging Initiative
ADRDA	Alzheimer's Disease and Related Disorders Association
ALS	Amyotrophic lateral sclerosis
ANOVA	Analysis of variance
APOE	Apolipoprotein E
APP	Amyloid precursor protein
BACE1	β-site amyloid precursor protein cleaving enzyme 1
BIN1	Bridging integrator 1 protein
BIOFINDER	Biomarkers for Identifying Neurodegenerative Disorders Early and Reliably
CCL2	C-C chemokine ligand 2
CHI3L1	Chitinase-3-like protein 1
CJD	Creutzfeldt-Jakob disease
CLU	Clusterin
CNS	Central nervous system
CR1	Complement component (3b/4b) receptor 1
CSF	Cerebrospinal fluid
DIAN	Dominantly Inherited Alzheimer Network
DLB	Dementia with Lewy bodies
DMT	Disease-modifying therapy
ELISA	Enzyme-linked immunosorbent assay
EOAD	Early-onset Alzheimer's disease
FAD	Familial Alzheimer's disease
FTD	Frontotemporal dementia
GFAP	Glial fibrillary acidic protein
HFABP	Heart fatty acid binding protein
IWG	International Working Group
LOAD	Late-onset Alzheimer's disease
MCI	Mild cognitive impairment
MCI-AD	Mild cognitive impairment due to Alzheimer's disease
MCP-1	Monocyte chemotactic protein 1

MMSE	Mini-Mental State Examination
NFL	Neurofilament light protein
Ng	Neurogranin
NIA-AA	National Institute on Aging and Alzheimer's Association
NINCDS	National Institute of Neurological and Communicative Disorders and Stroke
NMDA	*N*-methyl-D-aspartate
NSE	Neuron-specific enolase
PD	Parkinson's disease
PET	Positron emission tomography
PICALM	Phosphatidylinositol-binding clathrin assembly protein
PSEN1	Presenilin 1
PSEN2	Presenilin 2
PSP	Progressive supranuclear palsy
P-tau	Phosphorylated tau
ROC	Receiver operating characteristic
SAD	Sporadic Alzheimer's disease
sAPPα	α-Cleaved soluble amyloid precursor protein
sAPPβ	β-Cleaved soluble amyloid precursor protein
SD	Standard deviation
SEM	Standard error of the mean
sMCI	Stable mild cognitive impairment
TREM2	Triggering receptor expressed on myeloid cells 2
T-tau	Total tau
VaD	Vascular dementia
VLP-1	Visinin-like protein 1
YKL-40	Chitinase-3-like protein 1 (CHI3L1)

Introduction

Alzheimer's disease

Historical background

Alois Alzheimer (1864 – 1915) was a clinical psychiatrist and neuropathologist practising at the Community Hospital for Mental and Epileptic Patients (*Städtische Anstalt für Irre und Epileptiker*) in Frankfurt am Main, Germany around the turn of the last century. In the autumn of 1901, he investigated a newly admitted 50-year-old female patient named Auguste Deter, who presented with mainly psychiatric symptoms such as paranoid psychosis and aggressiveness, but also memory disturbances and progressive confusion that were deteriorating at a relatively quick pace [1]. Alzheimer became deeply interested in this case and started documenting his clinical findings very detailed and extensively during the course of Auguste's hospitalisation [2]. In 1902, Alzheimer moved to Munich where a large university hospital for psychiatry was being established, including a (for the time) modern histopathological laboratory, which Alzheimer became head of [1]. In 1906, Alzheimer's previous employer, the director of the Frankfurt Community hospital informed about the passing of Auguste Deter, who had remained hospitalised up until her death at age 55. An autopsy was arranged and brain material for histological investigation was sent to Alzheimer [2]. Those samples turned out to be the basis for the first description of the alterations later known as plaques and neurofibrillary tangles, the histopathological hallmarks of Alzheimer's disease. Emil Kraepelin, one of Alzheimer's co-workers in Munich, was excited about these findings, which had never been described before, and encouraged Alzheimer to present them at a conference. So, in November 1906, Alzheimer travelled to Tübingen and gave a lecture on this unusual case study at the 37th Meeting of South-West German Psychiatrists (*37. Versammlung Südwestdeutscher Irrenärzte*). One year later, the report was also published in a German medical journal [3]. Although the case report did not receive much attention at its initial presentation, Emil Kraepelin, who was an authority at the time, included it in the 8th edition of his textbook on psychiatry published in 1910 [4], thereby coining the term "Alzheimer's disease" (AD) already during Alois Alzheimer's lifetime.

However, the disease, and with it Alzheimer's name, was more or less forgotten during most of the 20th century. It was not until the 1980s that modern Alzheimer research became reignited with the discovery of β-amyloid as the main component of senile plaques leading to the drafting of the so-called amyloid cascade hypothesis (more on that under *Pathophysiology* below). Shortly thereafter, in the 1990s, the discovery of the first pathogenic mutations also shed light on the genetic background of the disease (see chapter *Genetics of Alzheimer's disease*).

Clinical presentation

AD is characterised by a lengthy disease course with slowly deteriorating cognitive functions over many years, up to two decades. In the initial stages, insidious episodic memory disturbances are typical, together with depressive symptoms and anxiety. In that stage, patients are still relatively well-functioning socially and can develop strategies to compensate for their cognitive shortcomings. This clinical phase is often referred to as mild cognitive impairment (MCI) [5, 6] or prodromal AD. Over time, as the illness progresses, the memory impairment becomes more severe and the patient can develop difficulties to carry out practical tasks (dyspraxia) as well as speech disorders (dysphasia). In addition, visuospatial functions can become impaired which limits the ability to orientate oneself in one's surroundings. Eventually, the decline in cognitive functions leads to complete social dependence in the final stage of the disease, where even psychiatric symptoms such as behavioural disorders, aggressiveness, confusion and psychotic episodes can be prevalent. Common causes of death include secondary conditions such as pneumonia, chronic heart failure and pulmonary embolism [7].

AD can be subgrouped into sporadic AD, comprising the vast majority of AD patients, and familial AD which is a rare form caused by specific point mutations in certain genes (more on that distinction in the chapter *Genetics of Alzheimer's disease*). Sporadic AD can be divided further into early-onset AD (age of onset ≤ 65 years) and late-onset AD (age of onset > 65 years) [8]. Since many elderlies with AD also have signs of vascular dementia (VaD), and since the distinction between these conditions can be troublesome, another clinical subgroup called mixed AD/VaD has been proposed and included into the international classification of diseases [8, 9].

Pathophysiology

Starting in the 1980s, the molecular basis of the histopathological hallmarks of AD has been the subject of extensive research. The main component of senile plaques has been identified as β-amyloid$_{1-42}$ (Aβ42) [10] originating from the large transmembranous amyloid precursor protein (APP) [11] by proteolytic cleavage in two positions. Cleavage in the extracellular domain is mediated by the β-site amyloid precursor protein cleaving enzyme 1 (BACE1), also referred to as β-secretase [12], whereas cleavage in the transmembranous domain is mediated by the γ-secretase complex [13, 14], resulting in Aβ42, as well as shorter Aβ forms (the most abundant of which is Aβ40) and the β-cleaved soluble form of APP (sAPPβ) [15]. Another, non-amyloidogenic pathway of APP-processing does exist, with APP being cleaved by α-secretase and γ-secretase, resulting in a small soluble fragment named p3 as well as the α-cleaved soluble form of APP (sAPPα), thereby precluding the formation of Aβ42 [16-18]. What distinguishes Aβ42 from other β-amyloid isoforms, such as the more prevalent 40 amino acid isoform Aβ40, is its aggregation properties. Aβ42 is more hydrophobic in nature and has a tendency to form Aβ oligomers [19] that further aggregate into larger insoluble structures that eventually result in the deposition of Aβ plaques [20].

The main component of the other histopathological feature of AD, the neurofibrillary tangles, has been identified as an abnormally truncated and phosphorylated variant of the tau protein, which is abundant in neurons as a microtubule-associated component of the cytoskeleton [21-23]. Tau plays an important role in stabilising microtubules and thereby facilitating axonal transport, which is vital for any nerve cell [24]. While a certain amount of tau phosphorylation may be of importance during brain development [25, 26], abnormal truncation and hyperphosphorylation of tau in the adult brain can lead to the formation of paired helical filaments and neurofibrillary tangles [27, 28], which disrupts the neuronal cytoskeleton and eventually causes synaptic dysfunction and neuronal cell death [29].

Based on this knowledge, different hypotheses for the pathogenesis of AD have been postulated over the years, the most prominent and most accepted of which being the so-called amyloid cascade hypothesis [30].

Amyloid cascade hypothesis

The amyloid cascade hypothesis states that abnormal accumulation of Aβ-containing plaques is the initiating pathogenic event and the primary cause of the

neurodegeneration seen in AD [31-33]. This event is believed to trigger a cascade of further pathological processes including the formation of neurofibrillary tangles, microglial activation, reactive astrocytosis, neuritic injury and eventually synapse loss and neuronal cell death [30, 34, 35]. It has been suggested that Aβ accumulation is a consequence of an imbalance between production of Aβ42 peptides and clearance of named peptides from the brain [36]. Genetic studies have provided evidence for this since an abnormal overproduction of Aβ peptides has been described in cases of familial AD, where mutations in the *APP*, *PSEN1* or *PSEN2* genes are present [37, 38]. Moreover, the ε4 allele of the *APOE* gene, which is the most important genetic risk factor for sporadic AD, has been shown to increase Aβ aggregation and impair its clearance from the brain [39, 40]. On the other hand, the amyloid cascade hypothesis has also been called into question, not least since cerebral Aβ burden correlates poorly with the extent of cognitive dysfunction [41, 42].

Diagnosis

The first diagnostic criteria for AD were published in 1984 by a work group established in the US by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) together with the Alzheimer's Disease and Related Disorders Association (ADRDA) [43]. It defined the diagnosis of probable AD on the basis of clinical examination and neuropsychological tests. A definite AD diagnosis could only be made post mortem by histopathological evidence. More than 20 years later, these criteria were revised by the International Working Group (IWG) for New Research Criteria for the Diagnosis of Alzheimer's Disease spearheaded by Bruno Dubois, providing research criteria focusing on a clinical core of episodic memory impairment supplemented by at least one supportive biochemical or radiological feature, such as abnormal findings on brain MRI, specific patterns on PET imaging or abnormal CSF biomarkers [44]. In 2011, the NINCDS-ADRDA workgroup revisited their original criteria and revised them in a way that retained the general framework of probable AD but added CSF biomarkers and amyloid-PET findings as evidence of AD pathophysiology [45]. In 2014, the IWG also refined their criteria (now called IWG-2 criteria) by defining AD diagnosis as a specific clinical phenotype together with *in vivo* evidence of AD pathology (CSF or PET biomarkers as well as AD mutations). They also added criteria for the preclinical states of AD defined by the absence of a specific clinical phenotype together with *in vivo* evidence of AD pathology [46]. The latest attempt in providing research criteria was published in 2018 by Clifford A. Jack Jr. and colleagues called the

NIA-AA research framework [47]. These so-called ATN criteria take on a different approach by defining AD on the basis of biomarkers alone (both biochemical and imaging), reflecting three different types of pathologies: Aβ deposition (the A criterion), pathologic tau (the T criterion) and neurodegeneration (the N criterion). According to this concept, AD is defined as a biological construct by using evidence of its unique neuropathological changes rather than a specific clinical phenotype.

Treatment

Symptomatic therapies

The only currently approved drugs for the treatment of AD are symptomatic therapies that fall into two categories: inhibitors of acetylcholinesterase (AChE) and partial antagonists of the *N*-methyl-D-aspartate (NMDA) receptor. AChE-inhibitors aim at improving cholinergic neurotransmission by increasing the amount of acetylcholine available in the synaptic cleft [48]. Partial NMDA receptor antagonists modulate the effect of glutamate, which is beneficial since neurodegeneration in AD can lead to glutamatergic hyperactivity and activation of extrasynaptic NMDA receptors that may introduce noise in synaptic signalling [49]. However, none of these therapies can modify the underlying disease mechanisms or halt disease progression in AD.

Disease-modifying therapies

The search for disease-modifying therapies (DMTs) for AD, although ongoing for over a decade, has proven to be difficult, with numerous drug trials failing because of lack of desired effect or unacceptable adverse effects [50, 51]. There are currently no approved disease-modifying drugs for AD and no new AD treatment has been approved since the approval of memantine (an NMDA receptor antagonist) in 2003 [51].

In line with the amyloid cascade hypothesis, the main target for DMTs is Aβ with three different mechanisms tested so far: (A) decreasing Aβ production by inhibiting BACE1 [52] or γ-secretase [53], alternatively by activating α-secretase and thereby shifting the balance towards the non-amyloidogenic pathway [54, 55], (B) increasing Aβ clearance by active and passive immunisation approaches

[56-58] or Aβ-degrading enzymes [59], and (C) inhibiting amyloid oligomerisation and fibril formation [60, 61]. Analogous to Aβ, both immunotherapy [62] and anti-aggregation therapies [63] have been tested targeting tau, with the aim of reducing the amount of neurofibrillary tangle pathology.

In spite of the rather disappointing results in drug development so far, there are still hopes that a breakthrough might be around the corner with several drug trials awaiting conclusion in the upcoming years. Currently, there are fourteen studies targeting Aβ and one study targeting tau ongoing in phase III [64]. There are also hopes that shifting focus towards the preclinical phase of AD, by including more study participants in this early stage of the disease, might increase the chance of a positive outcome in drug trials [65].

Genetics of Alzheimer's disease

Even long before the first genetic variants associated with AD risk were described, there was mounting evidence for a genetic contribution to the disease. Clustering of AD was described both in the rare familial form of AD and in the more common sporadic form [66-68]. In familial AD, inheritance appears to follow an autosomal-dominant pattern, whereas a more complex multifactorial inheritance has been suggested for sporadic AD [69-71].

Familial Alzheimer's disease

Starting in the early 1990s, genetic studies were conducted focusing on families affected with familial AD (FAD), with the goal of finding genes that contribute to AD risk. The first gene to be identified was *APP*, encoding the amyloid precursor protein (APP) [31, 72, 73]. APP is localised on chromosome 21, which also provides a plausible explanation as to why patients with Down syndrome, carrying a duplication of chromosome 21, frequently develop cerebral plaque and tangle pathology in a similar fashion as in AD [74, 75]. Mutations in the *APP* gene can cause changes in the proteolytic cleavage of APP, favouring pathways that lead to the production of the amyloidogenic 42 amino acid isoform of Aβ (Aβ42), at the expense of other cleavage products that lack aggregation properties, such as the slightly shorter and more soluble Aβ40 [76].

Other mutations causing FAD have been identified in the *PSEN1* gene located on chromosome 14 [77] and in the *PSEN2* gene located on chromosome 1 [78, 79].

Those genes encode two proteins, presenilin 1 and presenilin 2, constituting parts of the large transmembranous enzyme complex γ-secretase, that has numerous known substrates [80], one of which being APP [14]. It is hypothesised that mutations in the *PSEN1* and *PSEN2* genes can lead to a partial loss-of-function of γ-secretase, so that the enzyme manages to cut at the 42nd and 40th amino acid of Aβ, but barely reaches the cleavage sites generating the shorter and more soluble Aβ forms [81]. As a curiosity, it can be mentioned that Auguste Deter, the patient upon whom Alois Alzheimer's first description of the disease was based, was a carrier of a *PSEN1*-mutation, as evidenced by genotyping performed on conserved tissue samples over a century after her passing [82]. However, there is still some uncertainty surrounding this case, since the reported mutation could not be validated in a subsequent study [83].

In each of those three genes (*APP*, *PSEN1* and *PSEN2*), many distinct mutations causing FAD have been identified with more than 150 mutations in *PSEN1* alone, most of which have been thoroughly documented and reviewed [84-86]. The Dominantly Inherited Alzheimer Network (DIAN) is an example of an international research initiative contributing to the continued identification of disease-causing mutations as well as conducting clinical trials and long-term observational studies including patients who have or are at risk for developing FAD (http://dian.wustl.edu/).

Sporadic Alzheimer's disease

In sporadic AD, which comprises more than 95% of all AD cases [87], the genetic aetiology is more complex. While there is a strong genetic component, the inheritance is not simply following a classic Mendelian pattern as is the case in FAD. Instead, a multifactorial aetiology has been suggested, with multiple low penetrance genetic polymorphisms that can increase (or decrease) the risk for disease onset. The first such susceptibility gene identified for sporadic AD was *APOE* [88, 89] located on chromosome 19 [90, 91], encoding apolipoprotein E (apoE), which functions as a lipid transporter in the brain and in blood [92]. ApoE exists in three isoforms (apoE2, apoE3 and apoE4) resulting from three polymorphisms in the *APOE* gene (*APOE* ε2, ε3 and ε4) which are differentiated from each other by single amino acid substitutions at positions 112 and 158, respectively [93, 94]. The ε3 allele is the most common of the three, whereas the less frequent ε4 allele is associated with a higher risk of developing AD in a dose-dependent manner [95, 96]. Heterozygous *APOE* ε4 carriers have a relative increase in risk that is approximately 3-fold, whereas homozygous *APOE* ε4

carriers can have up to 15-fold increase in risk compared to homozygous *APOE* ε3 carriers [97, 98]. Moreover, there is also an *APOE* ε4 dosage effect on the mean age of onset, which is lower in *APOE* ε4 carriers compared to non-carriers [95]. The ε2 allele, on the other hand, has been described as a protective factor for the development of sporadic AD [99, 100].

Apart from *APOE*, a number of other susceptibility genes for sporadic AD, as well as polymorphisms associated with a lower risk of developing AD, have been identified in genome wide association studies. For example, *CR1* located on chromosome 1 encoding the complement component (3b/4b) receptor 1 [101], as well as *BIN1* located on chromosome 2 encoding the bridging integrator 1 protein [102], are associated with a higher risk of developing AD. Examples for risk-lowering polymorphisms are *CLU* located on chromosome 8 encoding clusterin [101, 103], as well as *PICALM* located on chromosome 11 encoding the phosphatidylinositol-binding clathrin assembly protein [103]. Over a dozen more potential susceptibility genes for AD have been pinpointed in a large meta-analysis of genetic association studies featured in the AlzGene database (which is publicly available at www.alzgene.org) [104]. However, it should be noted that none of these associations are anywhere as strong as the one observed for *APOE*.

Biomarkers for Alzheimer's disease

Cerebrospinal fluid biomarkers reflecting the core pathologies of Alzheimer's disease have been on the radar for researchers for at least three decades, in particular the 42 amino acid isoform of β-amyloid (Aβ42) reflecting plaque pathology, and phosphorylated tau (P-tau) reflecting neurofibrillary tangle pathology. Together with total tau (T-tau), reflecting axonal neurodegeneration, these CSF biomarkers are often referred to as the core AD biomarker triad and they are well-established today and used widely in clinical practice to diagnose both manifest and incipient Alzheimer's disease. In more recent years, blood-based biomarkers for AD started to appear on the horizon, yielding mixed, but also some promising results. However, as of today, plasma biomarkers for AD have not yet made their entrance into everyday clinical routine use. A more detailed account of the various AD biomarkers is outlined below, according to the different pathologies they reflect.

Biomarkers for β-amyloid pathology

The major constituent of senile plaques, the 42 amino acid isoform of Aβ (Aβ42), is measurable in CSF and concentrations of this biomarker are lower in AD compared to controls [105], which has been verified in numerous studies [106]. The same can be observed in patients with mild cognitive impairment as well as in the preclinical phase of AD [107-110], and today Aβ42 is widely accepted as a robust measure of cerebral plaque pathology. The lower concentrations are due to the sequestration of Aβ42 in senile plaques, leaving only the soluble fraction of the protein detectable in CSF [111, 112]. A decrease in CSF Aβ42 concentrations can also be seen in dementia with Lewy bodies (DLB) [113] as well as secondary to CNS infections [114]. Other APP cleavage products, such as Aβ38, Aβ40, sAPPα and sAPPβ have been measured in the CSF of AD patients, albeit with no or only negligible differences when compared to controls [115-117], making those proteins less usable as biomarkers than Aβ42.

Unlike in CSF, it has been much more difficult to find reliable biomarkers for Aβ pathology in peripheral blood. While being measurable in plasma, concentrations of Aβ42 do not seem to reflect plaque pathology in the brain [118-120]. It was not until recently that correlations between plasma Aβ42 and CNS amyloidosis have been reported in studies using mass spectrometry-based methods [121] as well as ultrasensitive assays [122]. These results are promising, but they need to be replicated in further studies.

Biomarkers for neurofibrillary tangle pathology

Abnormally truncated and phosphorylated tau (P-tau) forms the main component of neurofibrillary tangles and can be measured in CSF using immunochemical assays detecting mid-domain epitopes of the protein [123, 124]. Concentrations are increased in AD [125] and CSF P-tau is regarded as the most specific AD biomarker, with herpes encephalitis and superficial CNS siderosis being the only other currently known conditions that give rise to elevated CSF P-tau levels [126, 127]. This is notable since other diseases that also feature neurofibrillary tangle pathology, such as frontotemporal dementia (FTD) and progressive supranuclear palsy (PSP), do not show the same increase in CSF P-tau concentrations seen in AD [128]. This has led to an alternative hypothesis regarding the mechanisms underlying the increase of T-tau and P-tau in CSF, namely that neurons exposed to Aβ pathology respond by increasing their secretion of tau (both total and phosphorylated forms) into the brain's interstitial fluid that communicates freely with the CSF [129, 130]. If this is correct, CSF T-tau and P-tau are not direct

biomarkers for neurodegeneration and tangle pathology in AD, but rather Aβ response markers that may be predictive of future neurodegeneration and tangle formation. In peripheral blood, no reliable biomarker for neurofibrillary tangle pathology has yet been identified.

Biomarkers for axonal neuronal degeneration

Neurodegeneration is prevalent in AD and one CSF biomarker that can be utilised to assess general axonal neurodegeneration is total tau (T-tau) [131], using assays measuring the total amount of tau released from dying neurons, irrespective of phosphorylation status [124, 132]. CSF concentrations of T-tau are increased in AD [124, 133] and, together with CSF Aβ42 and P-tau, it is now widely used as a biomarker for AD in clinical practice. However, it is not specific for AD – instead, elevated levels can frequently be seen in other neurodegenerative conditions, such as Creutzfeldt-Jakob disease (CJD) [134] and following stroke [135]. Among neurodegenerative dementias other than CJD, CSF T-tau is surprisingly AD-specific and hence not a general marker of neurodegeneration. This has led to an alternative hypothesis regarding the mechanism underlying CSF T-tau increase in AD (increased secretion in response to Aβ pathology, see above for details and references). Another CSF biomarker for axonal neurodegeneration is neurofilament light (NFL), which is present in long myelinated axons, thereby reflecting subcortical axonal damage when seen elevated in CSF [136]. AD patients show increased concentrations of NFL in CSF [137, 138]. However, this biomarker is not specific for AD and elevated levels of NFL have been observed in other conditions in which subcortical axonal neurodegeneration is prevalent, such as FTD and vascular dementia (VaD) [139-142] as well as in atypical parkinsonian disorders [143, 144], multiple sclerosis [145, 146], CJD [147, 148] and in amyotrophic lateral sclerosis (ALS) [149]. Apart from T-tau and NFL, other CSF biomarkers of neurodegeneration, for which increased concentrations in AD have been reported, are visinin-like protein 1 (VLP-1) [150] and heart fatty-acid binding protein (HFABP) [151]. Moreover, neuron-specific enolase (NSE) in CSF has been suggested as a biomarker for axonal neuronal loss in AD [152, 153], although measurement of NSE is known to be very susceptible to blood contamination [154].

Highly sensitive assays for measurement of both T-tau and NFL in peripheral blood have recently been developed [155]. Plasma NFL does seem to reflect subcortical axonal damage in the brain fairly well, both in AD and in other conditions with elevated CSF NFL concentrations [156, 157]. As far as plasma

T-tau is concerned, the correlations with levels measured in CSF are not as convincing compared with NFL, but nevertheless promising [158, 159].

Biomarkers for synaptic degeneration

Synaptic dysfunction is thought to be a common feature in AD, and it is suggested that early memory impairment in AD begins when synapses in certain brain regions, such as the hippocampus, are lost [160]. One CSF biomarker that reflects synaptic degeneration is neurogranin (Ng), a protein enriched in hippocampal neurons [161]. Increased CSF concentrations of Ng have been described in AD, but not in other neurodegenerative conditions, making it the most well-studied AD biomarker reflecting synaptic dysfunction or degeneration to date [162-167]. On the other hand, no blood-based biomarkers for synaptic degeneration have yet been discovered. While Ng has been measured in plasma, its concentrations were unable to distinguish between AD and healthy controls [168].

Biomarkers for glial activation

Activation of glial cells in the brain, both astrocytes and microglia, has mainly been linked to neuroinflammatory conditions, but also to AD [169, 170]. Astrocytes are glial cells that play an important role in repair mechanisms as well as forming part of the blood-brain-barrier, whereas microglia are macrophages that constitute the innate immune defence of the brain. A number of glial biomarkers have been reported as being increased in the CSF of AD patients, namely chitotriosidase [171, 172], soluble CD14 [173], chitinase-3-like protein 1 (CHI3L1), also known as YKL-40 [174-176] and the C-C chemokine ligand 2 (CCL2), also known as monocyte chemotactic protein 1 (MCP-1) [177, 178]. However, these increases are often less pronounced compared to those observed in neuroinflammatory conditions. Another commonly used biomarker for astroglial cell activation and damage, the glial fibrillary acidic protein (GFAP), has been seen elevated in the CSF of patients with multiple sclerosis [179], in herpes encephalitis [180] and following head trauma [181] and stroke [182]. Some studies have even reported elevated levels of GFAP in AD [183], whereas others have not [184]. More recently, the secreted ectodomain of the triggering receptor expressed on myeloid cells 2 (TREM2), which is selectively secreted from microglia in the CNS, has been reported to be increased in the CSF of AD patients [185-187], making this biomarker a promising and potentially more disease-specific addition to the glial biomarkers described above, although elevated levels have also been reported in multiple sclerosis [188]. In plasma, many of the

biomarkers for glial activation are measurable, but they do not seem to reflect CNS-related changes, indicating that they derive not only from the CNS but also from cells in the peripheral blood, which makes them less usable for diagnostic purposes. However, a slight increase of plasma YKL-40 in AD has been demonstrated in some studies [189].

Biomarkers for α-synuclein pathology

The presynaptic protein α-synuclein has been identified as the main component of the so-called Lewy bodies, which are aggregates seen in DLB and Parkinson's disease (PD) [190]. In those conditions, concentrations of α-synuclein in the CSF are typically decreased [143, 191]. On the contrary, in AD, while α-synuclein aggregates can be prevalent, CSF concentrations of α-synuclein have been reported to be increased, suggesting that this biomarker might also reflect nonspecific neurodegeneration [191-195]. According to this theory, decreased levels of CSF α-synuclein might reflect α-synuclein aggregation, while increased levels might indicate neurodegeneration, making it difficult to interpret this biomarker in cases where both of these pathologies are present [196]. Recently, promising results regarding the detection of pathological α-synuclein seeds in CSF from patients with PD and other synucleinopathies using real-time quaking-induced conversion RT-QuIC-based assays were published [197, 198]. These studies, if replicated, suggest that α-synuclein pathology could be detected in CSF in a similar manner as pathological prion proteins in CSF from patients with CJD. In peripheral blood, the high expression of α-synuclein in red blood cells limits its usability as a biomarker as well as making measurements in CSF more susceptible to blood contamination [199, 200].

Aims and objectives

The overall goal of this thesis is to ascertain the current state of biomarkers for Alzheimer's disease as well as to examine the association between biomarkers reflecting Alzheimer pathology and the *APOE* polymorphism.

More specifically, the aims of each paper are as follows:

Paper I:
To provide a comprehensive meta-analysis of the Alzheimer biomarker literature from 1984 (when the first diagnostic criteria for AD were proposed) up until 2014.

Paper II:
To examine the association between AD and the *APOE* polymorphism in a multicentre setting and to explore how this association is altered by biomarker assisted diagnosis making.

Paper III:
To test the hypothesis that the *APOE* polymorphism affects the diagnostic accuracy of biomarkers for AD in a multicentre setting.

Paper IV:
To examine how the *APOE* polymorphism affects biomarker concentrations in cognitively healthy individuals across all age groups in a multicentre setting.

Methods

Studies included in the meta-analysis

Search strategy

The objective of the search strategy employed for the meta-analysis in paper I was to cover biomarker related articles published between July 1st, 1984 (when the first diagnostic criteria for AD were proposed by McKhann et al. [43]) and June 30th, 2014. PubMed and Web of Science were used as search engines and only articles published in English have been considered. In order to be eligible for the meta-analysis articles must report data for at least one of the following biomarkers measured in either CSF or blood reflecting:

- Neurodegeneration: T-tau, NFL, NSE, VLP-1, HFABP
- APP metabolism: Aβ42, Aβ40, Aβ38, sAPPα, sAPPβ
- Neurofibrillary tangle pathology: P-tau
- Blood-brain barrier function: CSF to serum albumin ratio
- Glial activation: YKL-40, MCP-1, GFAP

In addition, all studies had to report comparisons in biomarker concentrations between AD patients and control subjects or between patients with mild cognitive impairment due to AD (MCI-AD) and patients with stable mild cognitive impairment (sMCI). Stable MCI was defined as MCI without progression to dementia during a follow-up time of at least 2 years. MCI-AD was defined as MCI with progression to AD at follow-up. Control subjects included both cognitively healthy volunteers and individuals admitted to hospital with non-neurological and non-psychiatric diagnoses (hospital controls).

Exclusion criteria

Articles were excluded if they:

- Did not contain an AD and a control cohort or
- Did not contain an MCI-AD and an sMCI cohort
- Had cohorts with fewer than 10 subjects

- Reported data in a format other than mean ± SD or mean ± SEM
- Had biomarker data from sources other than CSF or blood
- Had used non-quantitative methods
- Did not provide the diagnostic criteria used for AD or MCI
- Had cohorts representing a mix of diagnoses
- Had sMCI cohorts with less than 2 years follow-up time
- Had cohorts with subjects under the age of 18
- Lacked appropriately referenced analytical methods
- Contained data already published in a previous article
- Had control cohorts with an inflammatory, neurological or psychiatric diagnosis

Data collection

All data were extracted from the articles by a reading team of ten researchers and then double-checked for accuracy independently by two researchers. Results were curated from cross-sectional studies as well as baseline measurements from longitudinal studies. If the same measurements were used in multiple publications, e.g. data from large initiatives such as the North American Alzheimer's Disease Neuroimaging Initiative (ADNI), only the first article for each biomarker was included in the meta-analysis. In case of longitudinal studies using the same baseline measurements in multiple publications, the study with the longest follow-up time was chosen to be included. If the data was presented in a format not suitable for inclusion in the meta-analysis, the corresponding authors were approached and asked to provide their data as either mean ± SD or mean ± SEM. In studies with multiple control groups, the most cognitively healthy control cohort was used.

Participants and sampling

Cohorts

Papers II and III used the same cohort with a total of 1345 subjects from four different centres in Sweden, Finland and Germany. The cohort consisted of 251 control subjects, 399 patients with sMCI, 287 patients with prodromal AD

(MCI-AD), 309 AD patients and 99 patients with dementias other than AD. The follow-up for the sMCI subjects was at least 2 years.

In paper III, we included an additional cohort consisting of 105 cognitively healthy younger subjects below the age of 35 from one centre in Gothenburg, Sweden. Those same subjects were also included in the large cohort of cognitively healthy individuals used in paper IV (see below).

In addition to the above described cohorts, paper III included two further cohorts that had undergone PET imaging; one consisting of 118 MCI-patients from 3 memory clinics in Sweden, and one comprising 53 subjects from the ADNI database.

In paper IV, we included a cohort consisting of 716 cognitively healthy subjects aged 17 to 99 years from seven centres in Sweden, Finland, Germany and Italy. The majority of these individuals were healthy volunteers. One subcohort also contained 138 patients with bipolar disorder without any cognitive impairment.

Sampling

CSF samples were obtained by lumbar puncture in the L3/4 or L4/5 interspace, collected in polypropylene tubes and stored frozen at –80°C until analysis. Long-term stability of CSF biomarkers for AD has previously been confirmed to be satisfactory under those circumstances [201]. The majority of the biomarker measurements used in paper II, III and IV were performed at the Clinical Neurochemistry Laboratory in Mölndal, Sweden whereas the remaining samples were analysed at different laboratories close to the centres that participated in the studies (Kuopio, Finland; Munich, Germany and Perugia, Italy). Pre-analytical sample handling was not actively standardised prior to sample collection but all participating centres and laboratories were part of the tightly inter-connected and collaborative BIOMARKAPD network that developed common pre-analytical standard operating procedures around the time of the studies, which speaks against consequential variation in this regard [202]. Nevertheless, centre harmonisation was needed, please see below for more information regarding this.

Analytical methods

CSF analyses

CSF concentrations of T-tau, P-tau and Aβ42 in paper II, III and IV were measured using commercially available sandwich enzyme-linked immunosorbent assays (ELISA) [105, 123, 124]. Part of the samples were analysed using a multiplex semiautomated platform (xMAP Luminex AlzBio3) [203]. All analyses were carried out by experienced laboratory technicians who were unaware of the clinical diagnoses.

Data normalisation

It is known that there is considerable inter-laboratory variability in CSF biomarker measurements across different sites [204]. Therefore, since all biomarker measurements used in paper II, III and IV originated from multiple centres, a normalisation procedure was necessary in order to make biomarker concentrations comparable across participating sites. This was approached by defining the largest centre cohort in each study as the reference group. Factors were then calculated between the *APOE* ε4-negative controls from each participating centre and the *APOE* ε4-negative controls in the reference group. These factors were applied to all data, hence relating biomarker concentrations in the different centres to those in the reference group. Similar normalisation measures have been previously used in other multicentre settings [108].

APOE genotyping

Genotyping for the *APOE* gene in paper II, III and IV was performed using allelic discrimination technology in order to define the single nucleotide polymorphisms of *APOE* (ε2, ε3, ε4) on each allele. Study subjects were grouped into *APOE* ε4-negative (*APOE* ε4 –/–) lacking the ε4 allele, heterozygous *APOE* ε4-carriers (*APOE* ε4 +/–) carrying one copy of the ε4 allele, and homozygous *APOE* ε4-carriers (*APOE* ε4 +/+) carrying two copies of the ε4 allele.

PET analysis

One cohort used in paper III underwent PET scanning of the whole brain using [^{18}F]flutemetamol as a tracer [205]. All scans were conducted at two centres in southern Sweden.

Statistical analyses

Meta-analysis

Results in paper I were presented as ratios of the mean biomarker concentrations between AD and controls, and between MCI-AD and sMCI, respectively. This measure is known as fold change and it was used in the meta-analysis to tackle inter-laboratory variability with respect to cut-off points and analytical assays [204]. A ratio above one indicates higher biomarker concentrations in the patient group, whereas conversely a ratio below one indicates higher biomarker concentrations in the control group. The delta method was used to calculate the standard error of the ratio between the mean values [206]. Publication bias was assessed with funnel plots. The meta-analysis performed was a random effects meta-analysis with the method of DerSimonian & Laird with the estimate of heterogeneity taken from the inverse-variance fixed-effect model [207].

Comparisons of biomarker concentrations

In paper III, the Mann-Whitney test for independent samples was used for pairwise comparisons of biomarker concentrations both between and within the diagnostic groups. Comparisons between more than two groups were done using a Kruskal-Wallis test for several independent samples. In paper IV, one-way analysis of variance (ANOVA) for several independent samples was used to compare biomarker concentrations between *APOE* ε4 carrier groups. The Pearson's chi-squared test was used to compare *APOE* genotype frequencies between healthy volunteers and patients with bipolar disorder.

ROC analysis

In paper II, receiver operating characteristic (ROC) analysis was used in order to determine cut-off points for T-tau, P-tau and Aβ42, comparing biomarker

measurements between AD patients and healthy controls followed by finding the maximum for Youden's index [208] based on the results from that ROC analysis. The resulting cut-off points were remarkably close to previously determined reference limits [209].

In paper III, the area under the ROC curve was calculated for all biomarkers and separately for each *APOE* ε4 carrier group in AD patients compared to healthy controls, as well as in MCI-AD patients compared to sMCI.

Regression models

In paper III, multiple backward stepwise binary logistic regression was used to study associations between clinical diagnosis and biomarker concentrations, age, sex and *APOE* ε4 carrier status. Analysis of covariance was used to study the association between Aβ42 concentrations and *APOE* ε4 carrier status when stratifying for [^{18}F]flutemetamol uptake on the PET-scans.

In paper IV, the trajectory of CSF Aβ42 with respect to age in different *APOE* ε4 carrier groups was modelled using restricted cubic splines and ordinary least squares regression. Age at initial decline of CSF Aβ42 was defined as the maximum Aβ42 concentration prior to a monotone descent with increasing age.

Results and discussion

Meta-analysis of the biomarker literature

The initial search conducted for the meta-analysis in paper I generated 3500 articles from PubMed (after removal of duplicates) as well as 624 articles identified from Web of Science. After removal of articles not fulfilling the inclusion criteria, 585 articles remained which were assessed for eligibility by the reading team. After thorough review of these studies, a further 354 articles had to be removed due to the exclusion criteria specified in the methods section, which left 231 articles for inclusion in the systematic review and meta-analysis.

Comparing AD patients to control subjects

Established AD biomarkers in CSF

The three CSF biomarkers commonly considered as the core biomarker triad for AD (T-tau, P-tau and Aβ42) all showed statistically significant differences between AD patients and controls with good effect sizes.

Data on T-tau in CSF was reported by 151 studies including a total of 11341 AD patients and 7086 control subjects. All comparisons from these studies, without a single exception, resulted in AD to control ratios above one for CSF T-tau, with an average ratio of 2.54.

For CSF P-tau, data from studies using methods recognising single or multiple detection epitopes were combined resulting in 89 studies including a total of 7498 AD patients and 5126 control subjects. As for T-tau, all comparisons for CSF P-tau resulted in AD to control ratios above one, with an average ratio of 1.88.

For CSF Aβ42, studies using methods recognising either the 1-42 or the x-42 detection epitope of Aβ were included resulting in 131 studies including a total of 9949 AD patients and 6841 control subjects. Apart from one single study [210], all

comparisons for CSF Aβ42 resulted in AD to control ratios below one, with an average ratio of 0.56.

CSF biomarkers of neurodegeneration (other than T-tau)

In contrast to T-tau, which is an integral part of the well-established AD biomarker triad, considerably fewer studies were available investigating other CSF biomarkers of neurodegeneration with respect to AD.

Data on NFL in CSF was reported by nine studies with comparisons resulting in an average AD to control ratio of 2.35. For NSE in CSF, data from seven studies yielded an average AD to control ratio of 1.47. Four studies compared CSF concentrations of VLP-1 between AD patients and control subjects with an average ratio of 1.46. HFABP in CSF was reported by five studies with an average AD to control ratio of 1.39.

All effect sizes of CSF biomarkers of neurodegeneration were statistically significant when comparing AD patients with control subjects.

CSF biomarkers of glial activation

Six studies reported data on YKL-40 in CSF with elevated concentrations in AD patients compared to controls, yielding a statistically significant average ratio of 1.28. Data on MCP-1 in CSF were reported by three studies showing a statistically significant difference with elevated concentrations in AD patients compared to controls. However, the average effect size for MCP-1 was minor (1.12). Two studies investigated the astroglial marker GFAP in CSF without any significant differences between AD patients and controls.

Biomarker of blood-brain-barrier function

The CSF to serum albumin ratio is commonly used to assess blood-brain-barrier function. The meta-analysis identified 20 studies reporting data on the albumin ratio in AD patients compared to controls resulting in a statistically significant difference with higher CSF to serum albumin ratio in AD, albeit with a very small average effect size of merely 1.10.

CSF biomarkers of APP metabolism (other than Aβ42)

Sufficient data for four different APP cleavage products in CSF, besides Aβ42, were available for inclusion in the meta-analysis. The only one of these to reveal a statistically significant difference between AD patients and controls, albeit with a minor average effect size, was CSF Aβ40. It was reported by 25 studies with comparisons yielding an average AD to control ratio of 0.94. CSF Aβ38 was analysed in eight studies without any significant difference between AD patients and controls. The same was true for CSF sAPPα and sAPPβ analysed in nine and ten studies, respectively.

Table 1 below shows a summary of all CSF biomarkers comparing AD patients and controls included in the meta-analysis.

matrix	*biomarker*	*studies*	*effect size*	*95% CI*	*p-value*
CSF	T-tau	151	**2.54**	2.44-2.64	*P<0.0001**
CSF	P-tau	89	**1.88**	1.79-1.97	*P<0.0001**
CSF	Aβ42	131	**0.56**	0.55-0.58	*P<0.0001**
CSF	NFL	9	**2.35**	1.90-2.91	*P<0.0001**
CSF	NSE	7	**1.47**	1.08-2.00	*P=0.014**
CSF	VLP-1	4	**1.46**	1.31-1.62	*P<0.0001**
CSF	HFABP	5	**1.39**	1.24-1.57	*P<0.0001**
CSF	YKL-40	6	**1.28**	1.23-1.35	*P<0.0001**
CSF	GFAP	2	**1.12**	0.58-2.15	*P=0.736*
CSF	MCP-1	3	**1.12**	1.06-1.18	*P<0.0001**
CSF/serum	Albumin ratio	20	**1.10**	1.01-1.20	*P=0.035**
CSF	Aβ40	25	**0.94**	0.90-0.99	*P=0.019**
CSF	Aβ38	8	**0.99**	0.88-1.12	*P=0.891*
CSF	sAPPα	9	**1.03**	0.93-1.14	*P=0.554*
CSF	sAPPβ	10	**1.02**	0.95-1.09	*P=0.605*

Table 1. CSF biomarkers for AD comparing AD patients to control subjects.
The effect size represents the ratio of the mean biomarker concentration between AD patients and control subjects presented with 95% confidence interval. P-values with asterisk [*] denote statistical significance.

Plasma biomarkers

The literature search generated sufficient data for seven plasma biomarkers to be included in the meta-analysis. The only one of those to reveal a statistically significant difference was plasma T-tau with elevated levels in AD compared to controls but with a great and probably assay-dependent variation across studies. It was reported in six studies yielding an average AD to control ratio of 1.95. Another plasma biomarker that showed an equally large effect size was YKL-40, which was analysed in three studies. However, the confidence interval was fairly wide, and the difference did not reach statistical significance.

Unlike the convincing findings of Aβ42 in CSF, plasma Aβ42 did not show any statistically significant differences between AD patients and controls, based on data from 22 studies. The same is the case for plasma Aβ40, which was analysed in 21 studies.

None of the other plasma biomarkers showed any significant differences between AD and controls. Plasma NSE was analysed in three studies, plasma HFABP in two studies and plasma MCP-1 in six studies.

Table 2 below shows a summary of all plasma biomarkers comparing AD patients and controls included in the meta-analysis.

matrix	*biomarker*	*studies*	*effect size*	*95% CI*	*p-value*
Plasma	Aβ42	22	**1.04**	0.96-1.12	*P=0.321*
Plasma	Aβ40	21	**1.04**	0.98-1.11	*P=0.167*
Plasma	T-tau	6	**1.95**	1.12-3.38	*P=0.018**
Plasma	NSE	3	**1.00**	0.86-1.17	*P=0.992*
Plasma	HFABP	2	**1.05**	0.83-1.33	*P=0.692*
Plasma	YKL-40	3	**1.95**	0.99-3.84	*P=0.053*
Plasma	MCP-1	6	**1.00**	0.89-1.13	*P=0.986*

Table 2. Plasma biomarkers for AD comparing AD patients to control subjects.
The effect size represents the ratio of the mean biomarker concentration between AD patients and control subjects presented with 95% confidence interval. P-values with asterisk [*] denote statistical significance.

Comparing MCI-AD patients to sMCI subjects

Apart from comparing biomarker concentrations in AD patients versus controls, the search strategy employed for the meta-analysis also aimed at finding articles that report biomarker data on patients with prodromal AD, *i.e.*, patients with mild cognitive impairment at the time of sampling who later converted to Alzheimer's disease (MCI-AD), as well as patients with mild cognitive impairment who remained stable during a follow-up time of at least two years (sMCI). The search generated sufficient data on six CSF biomarkers as well as two plasma biomarkers to be included in the meta-analysis.

Established AD biomarkers in CSF

Comparing concentrations of the core AD biomarker triad in CSF between MCI-AD patients and sMCI subjects revealed similar results as the comparison between AD patients and controls, with all three biomarkers yielding statistically significant differences, albeit with somewhat smaller effect sizes.

Data on T-tau in CSF was reported by 12 studies. All comparisons from these studies resulted in MCI-AD to sMCI ratios above one for CSF T-tau, with an average ratio of 1.76. Likewise, all comparisons for CSF P-tau, curated from nine studies, revealed MCI-AD to sMCI ratios above one, with an average ratio of 1.72. For CSF Aβ42, data was reported by 12 studies resulting in an average MCI-AD to sMCI ratio of 0.67.

CSF biomarkers of APP metabolism (other than Aβ42)

Three studies reported data on CSF biomarkers of APP metabolism other than Aβ42 (namely Aβ40, sAPPα and sAPPβ) comparing MCI-AD patients to sMCI subjects. However, none of these comparisons yielded any statistically significant differences.

Plasma biomarkers

Three studies reported data on Aβ42 and Aβ40 in plasma compared between MCI-AD and sMCI. The meta-analysis revealed no statistically significant differences for plasma Aβ42. Conversely, plasma concentrations of Aβ40 did differ between

MCI-AD and sMCI, the effect size however was negligible with an MCI-AD to sMCI ratio very close to one (1.07).

Table 3 below shows a summary of all CSF and plasma biomarkers comparing MCI-AD patients and sMCI subjects included in the meta-analysis.

matrix	*biomarker*	*studies*	*effect size*	*95% CI*	*p-value*
CSF	T-tau	12	**1.76**	1.64-1.89	*P<0.0001**
CSF	P-tau	9	**1.72**	1.46-2.02	*P<0.0001**
CSF	Aβ42	12	**0.67**	0.63-0.73	*P<0.0001**
CSF	Aβ40	3	**0.98**	0.90-1.07	*P=0.715*
CSF	sAPPα	3	**1.09**	0.96-1.25	*P=0.195*
CSF	sAPPβ	3	**1.06**	0.87-1.28	*P=0.586*
Plasma	Aβ42	3	**0.81**	0.53-1.24	*P=0.324*
Plasma	Aβ40	3	**1.07**	1.03-1.10	*P=0.0002**

Table 3. CSF and plasma biomarkers for AD comparing MCI-AD patients to sMCI subjects.
The effect size represents the ratio of the mean biomarker concentration between MCI-AD patients and sMCI subjects presented with 95% confidence interval. P-values with asterisk [*] denote statistical significance.

Implications

To sum up, the biomarker performance of T-tau, P-tau and Aβ42 as well as NFL in CSF was significant with good effect sizes. NSE, VLP-1, HFABP and YKL-40 in CSF showed significant performance with moderate effect sizes. All other CSF biomarkers were either non-significant or significant with minor effect sizes. The only plasma biomarker to show significant performance was T-tau. In addition, the biomarker performance of T-tau, P-tau and Aβ42 in CSF was not only significant when comparing AD patients to controls, but also among patients with prodromal AD compared to stable MCI controls.

The results from this meta-analysis confirm unequivocally that the established AD biomarker pattern, *i.e.*, elevated CSF concentrations of T-tau and P-tau in combination with decreased CSF concentrations of Aβ42, is robustly associated with (both manifest and incipient) AD, which underlines that these biomarkers can and should be used generously in clinical routine. Moreover, increased CSF concentration of NFL is shown to be associated with AD, which indicates that subcortical axonal degeneration is present in AD. Measurement of NFL in CSF,

in conjunction with the already established AD biomarkers, could therefore be a useful addition in the diagnostic work-up of the disease.

Furthermore, increased CSF concentrations of NSE, VLP-1, HFABP and YKL-40 are associated with Alzheimer's disease, which is notable since none of these biomarkers reflects the core pathology of AD [211-215]. Instead, these biomarkers could be used as a measure of neurodegeneration and glial activation independently of Aβ42 and T-tau, which could prove to be useful in future clinical trials of drugs targeting tau- or amyloid-pathology.

It is true that some of the other CSF biomarkers investigated in this meta-analysis, namely Aβ40, MCP-1 and the CSF to serum albumin ratio, did show significant differences between AD patients and controls. However, their small effect size renders them useless as diagnostic biomarkers. The same is the case for the remainder of the CSF biomarkers, none of which showed any difference at all between AD patients and controls (GFAP, Aβ38, sAPPα and sAPPβ).

As far as biomarkers in plasma is concerned, none of the biomarkers of APP-metabolism showed any differences between AD patients and controls, which indicates that plasma levels of these metabolites do not reflect amyloid pathology in the brain. On the contrary, T-tau in plasma is shown to be capable of distinguishing between AD patients and controls, which could make it a desirable candidate in the future, especially due to the fact that the sampling procedure is far easier and more accessible for plasma compared to CSF. However, it should be noted that the findings are based on relatively few studies with large and probably assay-dependent variation, which necessitates further and larger studies to verify this association and determine the most reliable way to measure tau in plasma.

Limitations

The nature of this meta-analysis makes it necessary to handle data from a variety of studies using different methods and assays for biomarker quantification, which makes it impossible to compare absolute concentrations of the various analytes between studies. To tackle this inter-laboratory variability [204], we used a fold change approach by calculating ratios of biomarker levels between the diagnostic groups (AD versus controls and MCI-AD versus sMCI).

For comparisons between prodromal AD and sMCI patients, we only included sMCI cohorts with a follow-up time of at least 2 years with cognitive stability. However, that time period might still be too short to rule out progression to AD, particularly since it is known that decreased CSF Aβ42 concentrations can precede clinical onset of dementia by at least a decade [109, 110, 216]. As this might have an impact on our analysis, the comparisons between MCI-AD and sMCI with the somewhat smaller effect sizes of the core AD biomarkers in CSF have to be interpreted with caution.

The literature search, despite being exhaustive, cannot guarantee one hundred percent coverage and is also limited to articles written in English, which is why some eligible studies might have been missed. Moreover, studies reporting data in a format that is unsuitable for the meta-analysis could not be included unless authors provided the missing data upon request.

For the three core AD biomarkers in CSF, comparing AD patients to controls, funnel plots suggested publication bias, which is why the results need to be interpreted with some caution, although their consistency is strong, and heterogeneity is small. Conversely, the less well-studied biomarkers had no publication bias but suffered from larger heterogeneity, which might be due to smaller sample sizes and less established analytical assays.

The association between *APOE* ε4 and Alzheimer's disease

The association between the *APOE* ε4 allele and late-onset Alzheimer's disease has been known since the early nineties [95]. In paper II, we dived deeper into that topic in order to elucidate how strong that association is and, more interestingly, how it can be altered by biomarker-assisted diagnosis making, especially since the inclusion of biomarker measurements has been proposed in more recent research and diagnostic criteria [44, 45, 217]. To accomplish this, we used data from a large multicentre cohort, which is described in more detail in the methods section of this thesis.

Clinically diagnosed Alzheimer's disease

First, we merged all AD and MCI-AD patients into one clinical AD group. Correspondingly, we merged all remaining diagnostic groups (controls, sMCI subjects and subjects with dementias other than AD) into a single group designated non-AD. Comparing these two groups revealed that the *APOE* ε4 allele was overrepresented in the AD group and the odds ratio for a positive *APOE* ε4 carrier status (either one or two copies of the *APOE* ε4 allele) was 4.45. Comparing only AD patients to controls yielded an even higher odds ratio of 6.35. These results are expected and well in line with earlier studies including the AlzGene meta-analysis of *APOE* [104].

Biomarkers only

In the next step, we completely disregarded all clinical diagnoses and dichotomised the material solely based on biomarker data. For that purpose, we calculated cut-off points for each biomarker that achieved the best possible separation between AD patients and controls. More details on cut-off point determination can be found in the methods section of this thesis.

CSF Aβ42

All study participants were subgrouped into amyloid-positive (CSF Aβ42 < 546 ng/L) and amyloid-negative (CSF Aβ42 ≥ 546 ng/L) regardless of diagnostic group. Comparing these two groups yielded a remarkably high odds ratio for a positive *APOE* ε4 carrier status of 6.27.

CSF T-tau and P-tau

On the other hand, dichotomising the data according to CSF T-tau and P-tau, once again regardless of diagnostic group, gave lower odds ratios for the presence of *APOE* ε4 compared with clinical diagnosis only (2.92 for T-tau and 2.98 for P-tau, respectively).

Complete AD biomarker signature

Finally, we grouped together all study participants with a complete CSF biomarker signature indicative of AD, *i.e.*, decreased CSF Aβ42 combined with increased CSF T-tau and P-tau according to the predefined cut-off values and compared this group with all study subjects presenting a negative AD biomarker pattern. Note that once again all clinical diagnoses were completely ignored. In this comparison the association with the *APOE* ε4 allele was stronger than in pure clinical diagnosis with an odds ratio as high as 7.66.

Clinical diagnosis and biomarkers combined

Calculating odds ratios on study subjects presenting clinical diagnosis together with a concordant complete biomarker profile further strengthened the association between *APOE* ε4 and AD, with an odds ratio of 10.4.

Implications

These results confirm earlier findings on a strong association between the *APOE* ε4 allele and AD [95]. More importantly, it is remarkable that the *APOE* ε4 allele appears to be as strongly associated with amyloid pathology as with clinically diagnosed AD. Moreover, a complete biochemical AD pattern on its own, without any clinical information, shows a stronger association with *APOE* ε4 than a clinical AD diagnosis. Finally, combining clinical diagnosis with biomarker data results in an even stronger association with the *APOE* ε4 allele. Therefore, incorporating biomarker data into research and clinical criteria should provide higher diagnostic accuracy as opposed to clinical diagnosis alone.

Limitations

Since the data used in paper II originates from several different sites in a multicentre setting, the biomarker measurements had to be normalised to account for inter-laboratory variability [204]. In addition, the diagnostic algorithms used in the participating memory clinics are not harmonised against each other, although all used the same diagnostic criteria. The average follow-up time for the sMCI subjects was 3 years, which might be considered too short to completely rule out progression to AD [109].

APOE ε4 and the diagnostic accuracy of biomarkers for Alzheimer's disease

After studying the association between the *APOE* ε4 allele and AD in paper II, we were interested to find out to what extent the *APOE* ε2/ε3/ε4 polymorphism actually affects the concentrations of the core AD biomarkers in CSF, and consequently also their diagnostic performance, particularly since earlier studies have indicated decreased CSF Aβ42 concentrations in *APOE* ε4 carriers [218-221], arguing that the *APOE* genotype should be taken into account when using CSF Aβ42 as a biomarker for AD [221-224]. Moreover, we wanted to elucidate whether an association between the *APOE* genotype and CSF biomarkers depends on cortical Aβ status as measured by PET imaging. To accomplish these tasks, we designed a study (paper III) that used the same multicentre cohort used in paper II. Furthermore, we added two separate cohorts with subjects that had undergone PET imaging, one from the Swedish BIOFINDER study, and one from the large North American ADNI study.

CSF Aβ42 in relation to *APOE* genotype

In all diagnostic groups, the concentrations of Aβ42 in CSF were lower in *APOE* ε4 carriers compared to non-carriers in a gene dose-dependent manner, with heterozygous *APOE* ε4 carriers presenting lower Aβ42 concentrations than *APOE* ε4 non-carriers, and homozygous *APOE* ε4 carriers showing even lower Aβ42 concentrations than heterozygous *APOE* ε4 carriers. These findings confirm that the *APOE* ε4 carrier status does indeed affect CSF concentrations of Aβ42. However, at the same time CSF Aβ42 differed significantly between AD patients and controls, as well as between MCI-AD patients and sMCI subjects, even when analysing subgroups according to *APOE* ε4 carrier status separately.

ROC analysis revealed high diagnostic accuracy for CSF Aβ42 comparing AD patients versus controls in subjects with zero (*APOE* ε4 –/–) or one (*APOE* ε4 +/–) *APOE* ε4 alleles. The diagnostic accuracy in subjects with two *APOE* ε4 alleles (*APOE* ε4 +/+) was lower, which was largely due to the low number of homozygous controls. Performing ROC analysis comparing MCI-AD versus sMCI showed similar results.

Logistic regression models revealed that CSF Aβ42 and the *APOE* genotype were independent statistical predictors of AD diagnosis.

CSF T-tau and P-tau in relation to *APOE* genotype

Contrary to Aβ42, CSF levels of T-tau and P-tau within the diagnostic groups did not show the same dose-dependent differences with respect to *APOE* ε4 carrier status. However, as was observed for Aβ42, CSF concentrations of both T-tau and P-tau differed significantly between AD patients and controls, as well as between MCI-AD patients and sMCI subjects, irrespective of *APOE* ε4 carrier status.

Also, ROC analysis confirmed that the *APOE* genotype did not affect the diagnostic performance of either CSF T-tau or P-tau. As for Aβ42, the diagnostic accuracy of T-tau and P-tau among homozygous *APOE* ε4 +/+ individuals was somewhat lower.

Stratifying for cortical Aβ status

One of the cohorts used to relate CSF Aβ42 levels to amyloid PET comprised subjects who had undergone [^{18}F]flutemetamol PET imaging (taken from the BIOFINDER study). Individuals with positive [^{18}F]flutemetamol uptake had lower concentrations of CSF Aβ42, which is an expected finding. However, when analysing patients with positive or negative [^{18}F]flutemetamol uptake separately, no differences in CSF Aβ42 were found between *APOE* ε4 negative subjects and subjects carrying at least one *APOE* ε4 allele. When adjusting for cortical [^{18}F]flutemetamol uptake, no association between CSF Aβ42 and *APOE* ε4 carrier status remained. These results were also replicated using data from another cohort comprising subjects who had undergone [^{11}C]-PiB PET scans (taken from the ADNI database).

Implications

The study conducted in paper III clearly verified that the *APOE* ε4 allele is associated with lower concentrations of CSF Aβ42 in a dose-dependent manner, which is in line with findings from earlier studies [218-222]. However, all three core AD biomarkers in CSF were capable of distinguishing between AD patients and controls, as well as between MCI-AD patients and sMCI subjects, irrespective of *APOE* ε4 carrier status, and also retained their high diagnostic accuracy no matter which *APOE* ε4 carrier group was used for comparison. Furthermore, the study confirmed that CSF concentrations of Aβ42 and the *APOE* genotype are in fact independently associated with AD diagnosis. Overall, these findings strongly

emphasise the robust diagnostic performance of these biomarkers, without the need to consider the patient's genetic background when interpreting the results.

Moreover, the absence of an *APOE*-dependent effect on CSF Aβ42 when stratifying for cortical Aβ uptake, suggests that CSF Aβ42 actually reflects cortical amyloid pathology rather than the *APOE* ε4 carrier status, which further underlines that the *APOE* genotype does not need to be taken into account when using CSF Aβ42 as a biomarker for AD.

Limitations

As for paper II, the data used in paper III originates from several different sites in a multicentre setting, which means that the biomarker measurements had to be normalised to account for inter-laboratory variability [204]. In addition, the diagnostic algorithms used in the participating memory clinics are not harmonised against each other, although all used the same diagnostic criteria. The average follow-up time for the sMCI subjects was 3 years, which might be considered too short to completely rule out progression to AD [109]. A follow-up time of 5-10 years might be needed to fully verify that an MCI case is indeed stable [107]. Another pitfall of the study is the relatively low number of homozygous *APOE* ε4 carriers, particularly among controls, despite the large size of the total cohort, which makes comparisons of *APOE* ε4 +/+ subjects between diagnostic groups somewhat more difficult to interpret.

APOE ε4 and biomarkers for Alzheimer's disease in cognitively healthy individuals

In paper III, we included a small cohort with cognitively healthy individuals under the age of 35 in order to assess the association between the *APOE* ε4 allele and CSF Aβ42 concentrations in that particular group. In paper IV, we then further evaluated how the effect of *APOE* on CSF Aβ42 varies by age in a large multicentre cohort consisting solely of cognitively healthy subjects across all age groups.

CSF Aβ42 in relation to *APOE* genotype

Surprisingly, the gene dose-dependent effect of the *APOE* ε4 allele on CSF Aβ42 concentrations, that was clearly present in the large multicentre cohort used in paper III, was totally absent in the smaller cohort consisting of patients with bipolar disorder and healthy age-matched controls under the age of 35. This finding spawned another study (paper IV) in which we analysed cognitively healthy individuals across all ages. In the latter study, the gene dose-dependent effect of the *APOE* ε4 allele on CSF Aβ42 concentrations was once again clearly visible when analysing the whole cohort, which included individuals from 17 to 99 years of age. However, when dividing the cohort into tertiles according to age, the effect was absent in the lower tertile containing subjects aged 45 or younger.

CSF Aβ42 across different age groups

Using the large cohort with only cognitively healthy individuals from paper IV, we then modelled the trajectory of CSF Aβ42 concentrations across the different age groups. The estimated curves showed an initial upslope of CSF Aβ42 concentrations in *APOE* ε4 –/– and *APOE* ε4 +/– subjects followed by a steep descent. *APOE* ε4 +/+ subjects lacked the initial upslope and showed a descent in CSF Aβ42 concentrations already from an early age. The age at which CSF Aβ42 reaches its maximum before the initial descent kicks in was estimated at 50 years for *APOE* ε4 –/– and 43 years for *APOE* ε4 +/– subjects. The age of initial descent could not be estimated for individuals carrying two *APOE* ε4 alleles (*APOE* ε4 +/+) since they lacked the initial upslope.

Implications

The absence of the gene dose-dependent effect of *APOE* ε4 on CSF Aβ42 concentrations in younger individuals who are more likely to be free from cerebral amyloid pathology, speaks against a primary (not amyloid mediated) effect of *APOE* ε4 on CSF Aβ42. In other words, the *APOE* ε4 allele does not appear to modify CSF Aβ42 concentrations unless pre-existing amyloid pathology is present in the brain. On the other hand, some studies comparing CSF Aβ42 with amyloid PET imaging suggest that the first decline in CSF Aβ42 concentrations does not always give rise to widespread cerebral amyloid deposition [225-227]. Therefore, the age of initial CSF Aβ42 descent could be interpreted as the starting point for preclinical disturbances in amyloid homeostasis that later result in detectable amyloid accumulation. The results from paper IV indicate that these disturbances

occur at a relatively young age, and even considerably earlier in *APOE* ε4 carriers compared to non-carriers. Moreover, compared to another study in which *APOE* ε4 was associated with cognitive decline only after 50 years of age [228], the data from paper IV shows declining CSF Aβ42 concentrations in heterozygous *APOE* ε4 carriers already from 43 years of age. This suggests that there may be an early period with incipient build-up of amyloid pathology that occurs before cognitive impairment becomes apparent [229]. Taken together, the findings from paper IV pinpoint the very earliest effects *APOE* ε4 has on CSF Aβ42 and may therefore be of importance for early diagnostics and potential preventive measures against AD, not least since previous studies have shown that incipient amyloid pathology, even at this early stage, may have unfavourable effects on brain function and cognition [229-232].

Limitations

As was the case for paper II and III, the data used in paper IV originated from several different sites using different analytical assays, which required data normalisation to account for inter-laboratory variability [204], potentially increasing the variance of our estimates. Moreover, the relatively low number of homozygous *APOE* ε4 carriers, particularly in the age span between 85 and 100 years, rendered it difficult to estimate the effect of *APOE* ε4 homozygosity in the final part of the natural life span. In addition, between the age of 35 and 50, the data set lacked homozygous *APOE* ε4 +/+ carriers, making it impossible to estimate the trajectory as well as the age of initial descent of CSF Aβ42 in this subgroup.

Conclusions and outlook

A robust tetrad of biomarkers and possible new candidates

The results from the meta-analysis performed in paper I clearly confirm that the established CSF biomarkers for AD, namely T-tau, P-tau and Aβ42, along with NFL, can be used to robustly and reliably assist with AD diagnosis making in a clinical setting. What is even more promising is that other CSF biomarkers, although not reflecting the core pathology of AD, also surfaced as possible new candidates from this meta-analysis, namely NSE, VLP-1, HFABP and YKL-40. In plasma, T-tau has shown potential to be useful as a diagnostic marker for AD, which is of particular interest, since plasma is a much more accessible and therefore desirable matrix for biomarker analysis, compared to CSF.

All results from the meta-analysis published in paper I are also included in a database that is freely accessible online (www.alzforum.org/alzbiomarker). The database contains additional data curated from the original papers, such as mean age of the cohorts, MMSE scores [233] and disease duration. In addition, it provides interactive visuals that allow users to make their own comparisons and explore possible new candidate biomarkers. Most importantly, the database is updated continuously as new studies are published, thereby serving as a living and ever-growing resource for the research community to use. As of June 2018 (version 2.1), the database contains 37 meta-analyses covering 26 different biomarkers, using data from 1546 cohorts published in 283 papers.

Biomarkers can deliver high diagnostic accuracy irrespective of *APOE* genotype

It has been known since the 1990s that there is a strong association between the *APOE* ε4 allele and AD. The results from this thesis have not only confirmed this but also shown that the association between the *APOE* ε4 allele and AD pathology, measured by CSF Aβ42, T-tau and P-tau alone (disregarding all clinical data), is at least as strong, if not slightly stronger. Moreover, the results confirm earlier findings of an association between the *APOE* ε4 allele and lower concentrations

of CSF Aβ42 in age groups in which amyloid pathology is prevalent, even without manifest AD. However, this association does not blur the robust diagnostic performance of CSF Aβ42 (as well as T-tau and P-tau) since the results of this thesis clearly show that these biomarkers are strongly associated with AD diagnosis and cortical Aβ deposition independently of *APOE* genotype. One important implication of this is that the patient's genetic status does not need to be taken into account when interpreting AD biomarker measurements and the clinical cut-off concentration for CSF Aβ42 should therefore be the same for all *APOE* genotypes.

APOE ε4 influences amyloid metabolism even in cognitively healthy subjects

One of the most surprising findings of this thesis is that the dose-dependent effect of the *APOE* ε4 allele on CSF Aβ42 concentrations is present even in cognitively healthy subjects, but only in age groups who are more prone to amyloid pathology. On the other hand, the effect is absent in the very young who are more likely to be free from cerebral amyloid deposition. This speaks against a primary effect of apoE isoforms on CSF Aβ42 concentrations and suggests that there has to be a turning point at which the effect becomes detectable and which then could be interpreted as the very earliest sign of preclinical disturbances in amyloid homeostasis. The results from this thesis suggest that this process might start already in early middle age in *APOE* ε4 carriers and several years later, but still relatively early, in *APOE* ε4 non-carriers. Our results, however, cannot explain the molecular mechanisms behind the association between apoE and cerebral Aβ build-up, and those will need to be addressed in future studies.

Future directions

Biomarkers are still, and continue to be, a valuable tool in the diagnosis of Alzheimer's disease and other neurodegenerative disorders. Moreover, their importance as theragnostic markers for the development of disease-modifying drugs should not be underestimated. Following the failure of drug trials in recent years, the field is more and more shifting focus towards the preclinical phase of the disease where early therapeutic intervention is more likely to yield promising outcomes. Therefore, it will be crucial to be able to capture the very earliest biochemical signs of amyloid pathology, long before cognitive impairment becomes apparent. For this purpose, biomarkers could be used to select

appropriate subjects for inclusion in future drug trials, as well as to monitor treatment efficacy along the preclinical and clinical course of AD.

In addition, emerging new candidate biomarkers that do not necessarily reflect what is considered the core pathology of AD, could be utilised as amyloid- and tau-independent measures of disease activity in drug trials, as well as provide more clues on underlying disease mechanisms that are yet to be fully understood. Lastly, the development of reliable analytical assays for the measurement of AD biomarkers in plasma rather than in CSF will facilitate their use in a clinical setting, even in remote places where access to specialist memory clinics is not readily available.

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