Experimental Neurology 223 (2010) 334–346

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Review

Biological markers of amyloid β-related mechanisms in Alzheimer's disease

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ARTICLE INFO

Article history:
Received 23 March 2009
Revised 21 September 2009
Accepted 26 September 2009
Available online 6 October 2009

Keywords:
Alzheimer's disease (AD)
Alzheimer's Disease Neuroimaging Initiative (ADNI)
Amyloid β-peptide (Aβ)
Amyloid precursor protein (APP)
Biochemical markers
Biomarkers
β-Site APP-cleaving enzyme 1 (BACE1)
Cerebrospinal fluid (CSF)
Diagnosis
Drug development
Mild cognitive impairment (MCI)
Mechanism of action
Neurochemistry
Oligomers
Plasma
Pre-clinical
Prediction
Presymptomatic
Stratification
US Food and Drug Administration (FDA)
European Medicines Agency (EMEA)

ABSTRACT

Recent research progress has given detailed knowledge on the molecular pathogenesis of Alzheimer's disease (AD), which has been translated into an intense, ongoing development of disease-modifying treatments. Most new drug candidates are targeted on inhibiting amyloid β (Aβ) production and aggregation. In drug development, it is important to co-develop biomarkers for Aβ-related mechanisms to enable early diagnosis and patient stratification in clinical trials, and to serve as tools to identify and monitor the biochemical effect of the drug directly in patients. Biomarkers are also requested by regulatory authorities to serve as safety measurements. Molecular aberrations in the AD brain are reflected in the cerebrospinal fluid (CSF). Core CSF biomarkers include Aβ isoforms (Aβ40/Aβ42), soluble APP isoforms, Aβ oligomers and β-site APP-cleaving enzyme 1 (BACE1). This article reviews recent research advances on core candidate CSF and plasma Aβ-related biomarkers, and gives a conceptual review on how to implement biomarkers in clinical trials in AD.

Contents

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doi:10.1016/j.expneurol.2009.09.024

Introduction

We face a global epidemic of Alzheimer's disease (AD) as the world's population ages. In 2006, the worldwide prevalence of AD was 26.6 million, and by 2050 the prevalence will quadruple. The current worldwide cost related to dementia is approximately $160 billion (Wimo et al., 2006). Without a significant improvement in prevention and treatment of AD, our healthcare and socioeconomic systems will not be able to carry the financial burden of AD in the future. However, interventions that delay disease onset or progression by only 1 year would reduce the disease prevalence by more than 9 million cases in 2050. Effective strategies for preventing and treating AD are therefore urgently needed before the national economies are overwhelmed by the financial burden of this growing epidemic.

Intense research efforts over the last 3 decades have given detailed knowledge on the molecular pathogenesis of AD. AD is a complex progressive condition with sequentially interacting pathological cascades, including the aggregation of amyloid β (Aβ) with plaque development, hyperphosphorylation and aggregation of tau protein with formation of tangles, together with downstream processes such as inflammation and oxidative stress, all of which contribute to loss of synaptic integrity, effective neural network connectivity and progressive regional neurodegeneration (Blennow et al., 2006). Research advances from pathological, neurochemical and genetic studies give increasing support to the "amyloid cascade hypothesis" (Hardy and Selkoe, 2002), which states that an imbalance between the production and clearance or degradation or clearance of Aβ in the brain is the initiating event in AD, ultimately leading to synaptic and neuronal dysfunction and degeneration with subsequent cognitive disturbances (Fig. 1).

These research advances have been translated into several new drug candidates with disease-modifying potential, several of which are now evaluated in clinical trials (Wisniewski and Konietzko, 2008). This foreshadows a new era of causal mechanistic treatment beyond symptomatic therapy. This new type of disease-modifying drugs can be expected to be most effective if initiated very early in the disease process, before the neurodegenerative process is too severe. However, current diagnostic manuals, such as the DSM-IV and ICD-10, warrant dementia, i.e., an advanced stage and severity of the disease, to make a clinical diagnosis of AD. Thus, there is a great need for improved diagnostic tools. New research criteria for diagnosis of AD implementing biomarkers to allow early identification have recently been proposed (Dubois et al., 2007).

Novel concepts of disease-modifying treatment also challenge current approaches for drug development. Drug trials on clinically diagnosed AD cases employing outcome measures based on clinical rating scales will not be sufficient to identify an effect of the new type of drugs in short-term and small-medium sized clinical trials. Biomarkers may speed up this process by serving as alternative outcomes to clinical measures. More accurate outcomes may also be achieved by enriching the population with patients with a disease-specific biomarker pattern, thus minimizing the risk of including patients who do not suffer from AD.

Biomarkers for AD

This review is focused on biochemical markers for the amyloidogenic process in AD in cerebrospinal fluid (CSF) and plasma. We use the term "biomarker" in a general sense to describe any measurable neurochemical indicator that is used to assess the risk or presence of disease. Biomarkers may facilitate the ability to reliably diagnose AD in the very early and perhaps even pre-clinical disease stages. They may also provide objective and reliable measures of drug safety and disease-modifying treatment efficacy in clinical drug trials in AD. Since the neuropathological changes of AD likely precede symptoms by years or decades, and it may well be optimal to treat the neuropathology as early as possible, biomarkers of pre-clinical AD are likely to play a pivotal role in the development of the next generation of therapies.

Criteria for an ideal biomarker for AD have been proposed by a consensus group on molecular and biochemical markers of AD (authors, 1998). The key features of an ideal AD biomarker are that it should detect a fundamental feature of the neuropathology, and have a diagnostic sensitivity for AD exceeding 80% together with specificity above 80% for distinguishing AD from other dementias. It should also be reliable, reproducible, non-invasive, simple to perform, and inexpensive. Recommended steps to establish a biomarker include confirmation by at least two independent studies conducted by qualified investigators with the results published in peer-reviewed journals, and validation in neuropathologically confirmed cases. Beyond these criteria for early and accurate diagnosis, it would be especially useful if the biomarker could track natural disease progression as well as the beneficial effect of disease-modifying therapies.

To facilitate clinical drug development for AD, it is of particular importance to be able to make accurate diagnoses early in the disease process, and to have biochemical measures that reflect the pharmacodynamic effects of treatment. For these reasons the National Institute on Aging (NIA) commissioned a working group on biomarkers as part of its Alzheimer's Disease Neuroimaging Initiative (ADNI) (Frank et al., 2003). A wide range of biological measures with possible relevance to AD were considered and then classified into categories of "Feasible, core," "Feasible, non-core" and "Uncertain feasibility." Feasibility was determined by the availability of a validated assay for the biological measure in question, with properties that included high precision and reliability of measurement, where reagents and standards were well described. Core analytes were those judged by the group to have reasonable evidence for association with key mechanisms of pathology implicated in AD, while non-core analytes were felt to be less clearly connected with mechanisms of pathogenesis or neurodegeneration in AD.

Development of feasible, core biological markers of Aβ-related mechanisms in AD

Key neuropathological hallmarks of AD are amyloid plaques and neurofibrillary tangles (Braak and Braak, 1991; Thal et al., 2002).

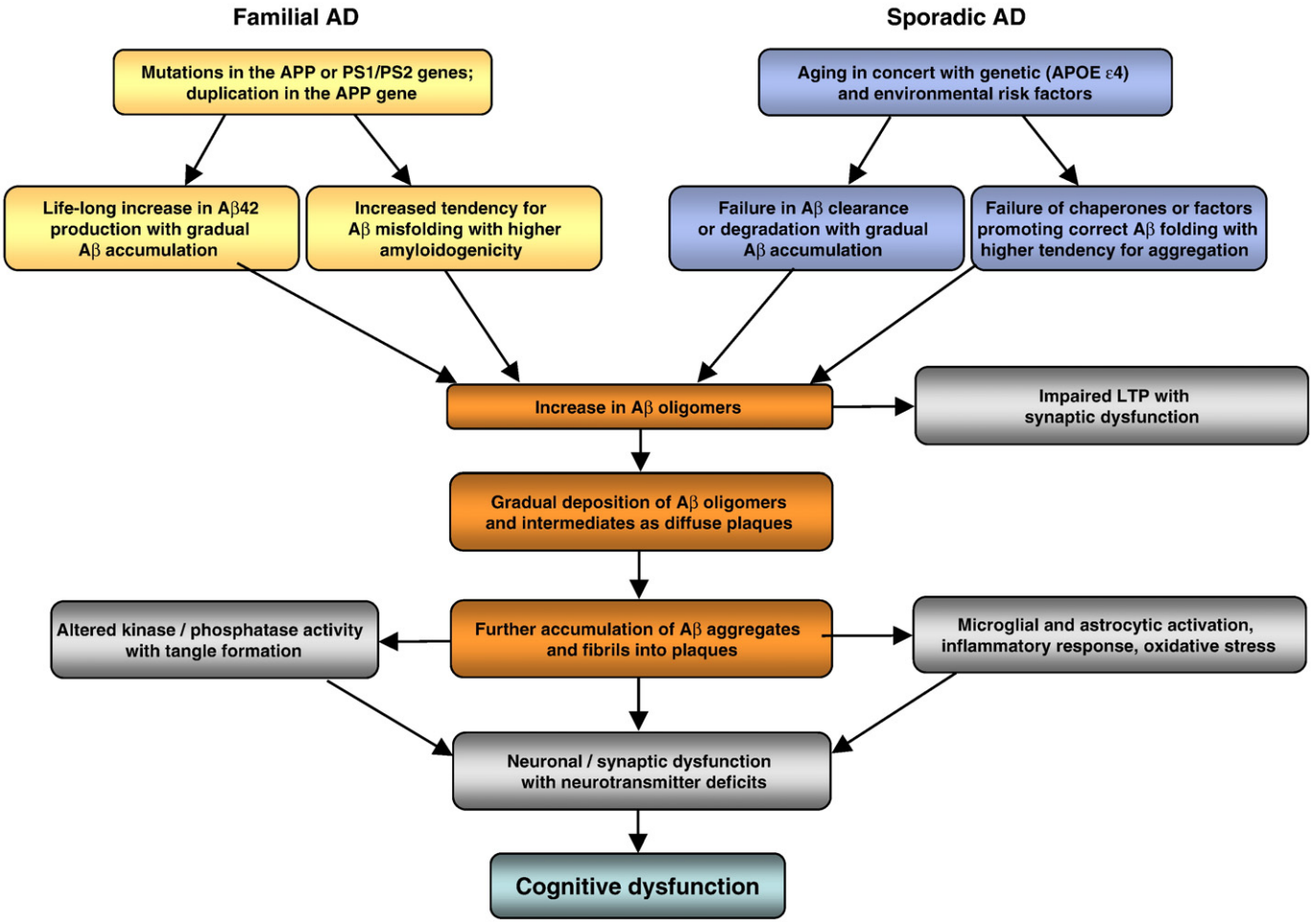

Fig. 1. The amyloid cascade hypothesis. According to this hypothesis, the central event in AD pathogenesis is an imbalance between Aβ production and clearance, with increased Aβ production in familial AD and decreased Aβ clearance in sporadic AD. Aβ oligomers could directly inhibit hippocampal LTP and impair synaptic function, in addition to the inflammatory and oxidative stress caused by aggregated and deposited Aβ. Tau pathology with tangle formation is regarded a downstream event, but may contribute to neuronal dysfunction and cognitive symptoms.

Amyloid plaques are relatively insoluble dense cores of 5-10 nm thick amyloid fibrils with a surrounding "halo" of dystrophic neurites, reactive astrocytes and activated microglia. The main proteinaceous component of amyloid plaques is the Aβ peptide. Aβ is not a single molecular entity, but rather is composed of a family of peptides produced by proteolytic cleavage of the type I transmembrane spanning glycoprotein Aβ precursor protein (APP) (Selkoe, 1999) (Fig. 2). Once released by proteolytic cleavage, the Aβ peptide may exist in solution and can be detected in CSF and plasma. This makes diverse species of Aβ peptides highly interesting and promising candidate biological markers (for review see Blennow and Hampel, 2003; Frank et al., 2003).

The pathogenic mechanisms that allow Aβ monomers to self-associate to form oligomeric and ultimately polymeric structures are not yet completely understood, but, as depicted schematically in Fig. 3, it is clear that Aβ can exist as monomers, dimers, oligomers, protofibrils, fibrils and fibrillar aggregates (Walsh and Selkoe, 2007). Moreover, the propensity for self-association of Aβ seems to depend on the peptide's primary sequence such that the Aβ42 variant, which makes up less than 10% of total Aβ, is more prone to aggregate than the more abundant Aβ40. Proposed mechanisms for Aβ-mediated "neurotoxicity" include structural damage to the synapse, oxidative stress, altered calcium homeostasis, induction of apoptosis, structural damage, chronic inflammation and neuronal formation of amyloid pores (Lashuel et al., 2002; Pratico, 2002; Selkoe, 1999).

Treatment trials with anti-amyloid drugs, such as active and passive immunization (Dodel et al., 2003) and β- and γ-secretase inhibitors (Wolfe, 2002), in AD patients will serve as the ultimate proof-of-concept regarding the validity of the amyloid cascade hypothesis. To this end, results from recent trials using biomarker candidates that signal effects of drugs targeting Aβ have been reported (Hock et al., 2003; Siemers et al., 2007). Advances in the development of core feasible neurochemical candidate biomarkers implemented as safety measures, enrichment and stratification variables as well as primary and secondary outcomes in clinical trials are currently paralleled by the development of multimodal structural and functional neuroimaging indicators (Hampel et al., 2008). These markers and technologies have been already implemented as secondary endpoints in trials aimed at abrogating the generation and accumulation of Aβ to make a claim for disease modification. They are currently under intense discussion by regulatory authorities such as the US Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) (Frank et al., 2003) in an effort to revise and update guideline documents. To be finally accepted by regulatory authorities as surrogate endpoints in clinical trials of potential AD modifying therapies both neurochemical and imaging biomarker candidates should respond to treatment, predict clinical response and be compellingly related to the pathophysiological processes, such as to the Aβ-related mechanisms of neurodegeneration in AD (Broich, 2007).

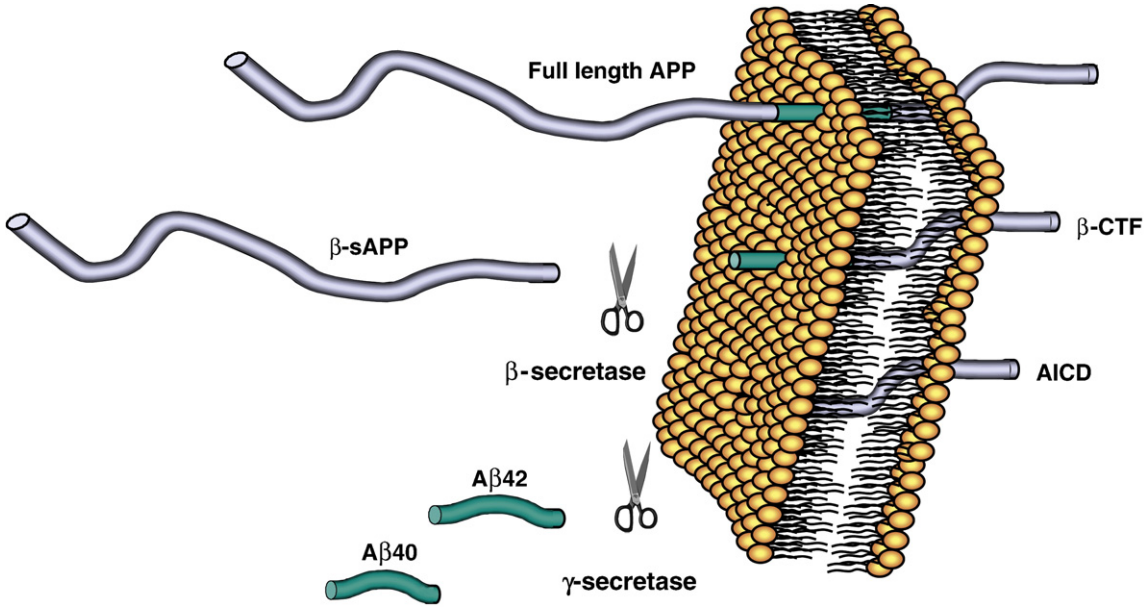

Fig. 2. Proteolytic cleavages of APP (the 770 amino acid isoform). APP processing is initiated by β-secretase after amino acid 671, which causes the secretion of the large β-sAPP molecule and the retention of a 99 residue C-terminal fragment (β-CTF). This fragment undergoes further cleavage by γ-secretase to release Aβ peptides terminating at residues 40 and 42, as well as several shorter Aβ isoforms.

Candidate biomarkers to reflect Aβ amyloidogenic processes in AD

This section of our article aims to provide an updated concise and comprehensive review on core candidate biomarkers with diagnostic potential and possible utility for monitoring the effects of disease-modifying therapies for AD. These biomarker candidates include APP isoforms, BACE1 protein level and activity, Aβ isoforms including Aβ42 and Aβ40, and autoantibodies against Aβ.

APP isoforms in CSF

APP is an integral membrane protein with a large extracellular domain, a single transmembrane region and a short cytoplasmic

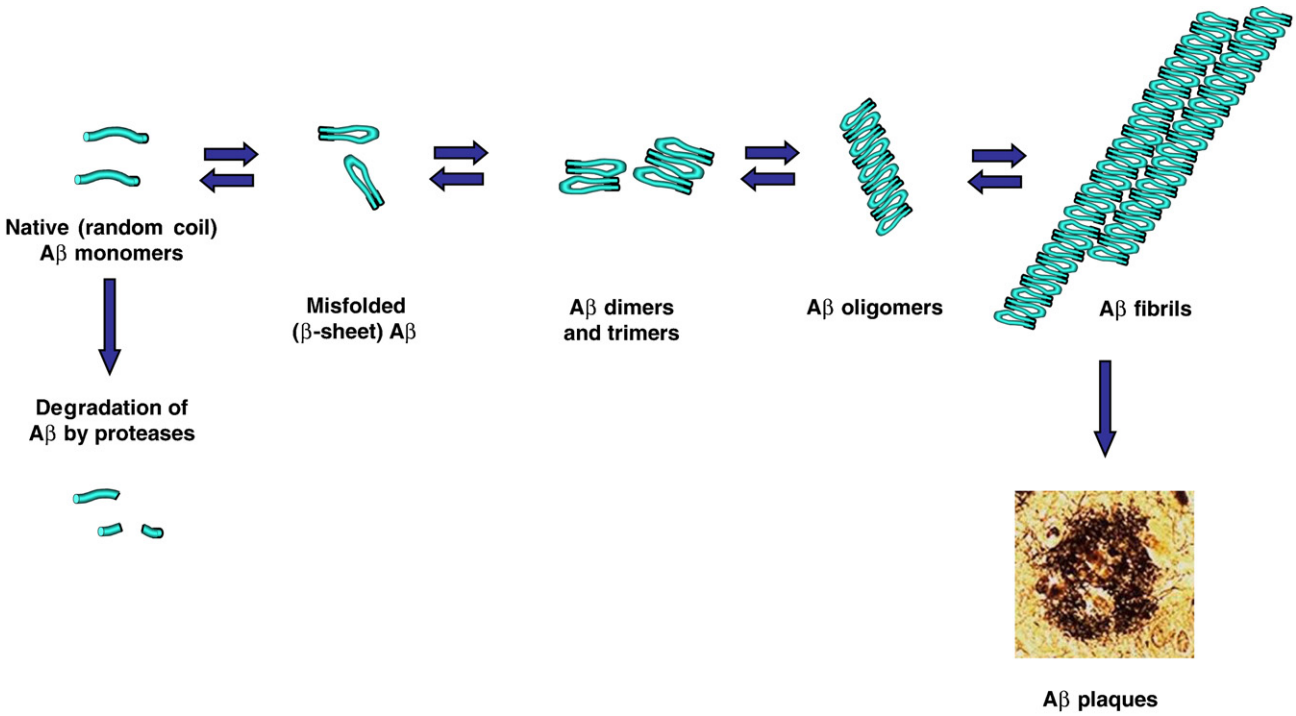

Fig. 3. Schematic model for amyloid β (Aβ) misfolding and aggregation. Soluble native protein is misfolded and associates in the form of oligomers and other intermediates that eventually give rise to fibrils. Potential opportunities for therapeutic intervention are shown in blue boxes.

domain (Fig. 2) (Haass, 2004). The biological function of APP remains uncertain. The γ-secretase released intracellular domain (ICD) of APP (AICD) has been suggested to function as a transcription factor, but genes regulated by AICD have not been unambiguously identified (Anliker and Muller, 2006). Extensive investigations using behavioural models (Conboy et al., 2005), neuronal cultures and APP knockout mice suggest that APP may serve as a receptor for and appears to play a role during axonal regeneration (Chen and Tang, 2006) and as a regulator of neural activity, connectivity, plasticity and memory (Conboy et al., 2005; Turner et al., 2003) and in the anterograde transport of vesicles along axons (Stokin et al., 2005), although it should be noted that considerable controversy exists regarding the last observation (Lazarov et al., 2005).

Large soluble APP (sAPP) fragments are present in CSF (Seubert et al., 1992); however, the results from studies on CSF levels of total, α- or β-cleaved sAPP in AD have been contradictory, ranging from an increase (Lewczuk et al., in press), to no significant change (Hock et al., 1998; Olsson et al., 2003; Zetterberg et al., 2008) or a slight decrease (Lannfelt et al., 1995; Palmert et al., 1990; Prior et al., 1991; Sennvik et al., 2000; Van Nostrand et al., 1992). In therapeutic studies, the CSF level of α-sAPP may be useful as a marker of α-secretase activation or β-secretase inhibition.

BACE1 protein level and activity in CSF

In 1999, several independent research groups published evidence demonstrating that a significant part of the β-secretase activity originates from an integral membrane aspartyl protease encoded by the *BACE1* gene (Hussain et al., 1999; Sinha et al., 1999; Vassar et al., 1999; Yan et al., 1999). Studies on *BACE1*-knockout mice harboring FAD mutations or being wild-type for the *PS* and *APP* genes indicate that BACE1 is indeed the major APP-cleaving β-secretase in the brain (Laird et al., 2005; Roberds et al., 2001). Given the fact that BACE1 knockout mice have a very mild phenotype, BACE1 has been considered a promising target for therapy. However, the recently identified role of BACE1 in myelination (Hu et al., 2006; Willem et al., 2006) and the finding that genetic ablation of BACE1 results in Schizophrenia-like changes (Savonenko et al., 2008) have raised some concerns about this approach.

Table 1
Performance of CSF tau and amyloid biomarkers for AD in the MCI or pre-clinical stage of the disease.

Reference	Year	Setting	Numbers included	AD-/dementia-associated change	Comment
(Andreasen et al., 1999b)	1999	Longitudinal MCI-control study	16 MCI-AD patients and 15 age-matched controls	Low CSF Aβ42, high CSF T-tau	Sensitivity 88%, specificity 80%
(Riemenschneider et al., 2002a)	2002	Longitudinal MCI study	28 MCI patients, 10 of whom developed AD	Low CSF Aβ42, high CSF T-tau	Sensitivity 90%, specificity 90%
(Zetterberg et al., 2003)	2003	Longitudinal MCI study	53 MCI patients, 22 of whom developed AD	Low CSF Aβ42, high CSF T-tau, high CSF P-tau181	Sensitivity 68%, specificity 97%, PPV 94%, NPV 81%
(Skoog et al., 2003)	2003	Population-based longitudinal cohort study	35 non-demented 85 year olds underwent LP and were followed for 3 years	Low CSF Aβ42	Low levels of CSF Aβ42 predicted progression to dementia
(Hampel et al., 2004)	2004	Longitudinal MCI-AD-control study	52 MCI patients, 93 AD patients and 10 controls	Low CSF Aβ42, high CSF T-tau	Sensitivity 59–83%, specificity 90–100%
(Herukka et al., 2005)	2005	Longitudinal MCI-control study	78 MCI patients, 23 of whom developed AD, 46 controls	Low CSF Aβ42, high CSF T-tau, high CSF P-tau181	Sensitivity 91%, specificity 56%
(Hansson et al., 2006)	2006	Longitudinal MCI study	137 MCI patients, 57 of whom developed AD	Low CSF Aβ42, high CSF T-tau, high CSF P-tau181	Sensitivity 95%, specificity 83%, PPV 81%, NPV 96%
(Herukka et al., 2007)	2007	Longitudinal MCI study	79 MCI patients, 33 of whom developed AD, 60 controls	Low CSF Aβ42, high CSF T-tau, high CSF P-tau181	Low levels of CSF Aβ42 predicted progression to AD
(Hansson et al., 2007)	2007	Longitudinal MCI study	137 MCI patients, 57 of whom developed AD	Low Aβ42/Aβ40 ratio	Sensitivity 87%, specificity 78%
(Li et al., 2007)	2007	Longitudinal control study	43 controls, 4 of whom developed MCI	High T-tau/Aβ42 ratio	Individuals with high ratio had higher *APOE* ε4 allele frequency and higher risk of progression to MCI
(Bouwman et al., 2007)	2007	Longitudinal MCI study	59 MCI patients, 30 of whom developed AD	Low CSF Aβ42, high CSF T-tau	Patients with abnormal values at baseline had higher risk of developing AD. Sensitivity and specificity missing.
(Brys et al., 2007)	2007	Longitudinal MCI-control study	65 MCI patients, 22 of whom developed AD, 21 controls	Low CSF Aβ42, low Aβ42/Aβ40 ratio, high CSF T-tau, high CSF P-tau231	Sensitivity 68–86%, specificity 60–91%
(Gustafson et al., 2007)	2007	Population-based longitudinal cohort study	55 cognitively healthy women underwent LP and were followed for 8 years	Low CSF Aβ42	Low levels of CSF Aβ42 predicted cognitive decline
(Stomrud et al., 2007)	2007	Longitudinal cohort study of healthy controls	57 cognitively normal controls underwent LP and were followed for 3 years	Low CSF Aβ42	Low levels of CSF Aβ42 predicted cognitive decline
(Ringman et al., 2008)	2008	Genetic case–control study	CSF biomarker results were compared in 7 asymptomatic carriers of familial AD (FAD)-associated mutations and four noncarriers	Low CSF Aβ42, low Aβ42/Aβ40 ratio, high CSF T-tau, high CSF P-tau181	Asymptomatic FAD mutation carriers had abnormal CSF biomarkers already in their 30s
(Shaw et al., 2009)	2009	Longitudinal multi-center study	196 MCI patients, 37 of whom developed AD	Low CSF Aβ42, high CSF T-tau, high CSF P-tau181	CSF T-tau/Aβ42 had a sensitivity of 89% for MCI cases with progression to AD
(Mattsson et al., 2009)	2009	Longitudinal multi-center study	750 MCI patients, 271 of whom developed AD	Low CSF Aβ42, high CSF T-tau, high CSF P-tau181	Sensitivity 83%, specificity 88% for MCI-AD versus controls; sensitivity 83%, specificity 72% for MCI-AD versus all MCI cases

Abbreviations: AD = Alzheimer's disease; MCI = mild cognitive impairment.

Recently, it was discovered that BACE1 activity can be measured in CSF. A first pilot study showed increased BACE1 activity in CSF from AD cases (Holsinger et al., 2004); this finding is consistent with the observation that BACE1 is upregulated in the AD brain and has been confirmed in subsequent studies, using different assay formats (Holsinger et al., 2006; Verheijen et al., 2006; Zhong et al., 2007). Importantly, recent studies show elevated BACE1 activity and protein levels in CSF of MCI patients (Zhong et al., 2007), and BACE1 activity in MCI cases that progress to AD with dementia (Zetterberg et al., 2008). These results suggest that upregulation of BACE1 may be an early pathogenic factor in AD. Interestingly, increased CSF BACE1 activity may be associated with the *APOE* ε4 allele in both AD and MCI subjects (Ewers et al., 2008). Taken together these results recommend CSF BACE1 activity as a promising potential candidate biomarker to monitor amyloidogenic APP metabolism in the CNS.

Aβ isoforms in CSF

To date, more than 30 different studies have been published analysing the diagnostic accuracy of the highly fibrillogenic 42 amino acid form of Aβ (Aβ42) in CSF (Blennow and Hampel, 2003). A 50% decrease in CSF Aβ42 control levels in AD patients has been found in most of the studies. The mean sensitivity and specificity to discriminate between AD and normal aging are both higher than 85% (Blennow, 2004). Other than in non-demented, aged individuals, normal CSF Aβ42 is found in psychiatric disorders, such as depression, and in neurological disorders such as Parkinson's disease and progressive supranuclear palsy (Blennow, 2004). However, a mild to moderate decrease in CSF Aβ42 may be found in a percentage of patients with frontotemporal dementia and vascular dementia (Hulstaert et al., 1999; Riemenschneider et al., 2002b; Sjogren et al., 2002; Sjogren et al., 2000), suggesting that the diagnostic performance of CSF Aβ42 alone in the discrimination between AD and other forms of dementia caused by different neurodegenerative mechanisms is insufficient. The reduced CSF level of Aβ42 in AD is believed to be caused by deposition of Aβ42 in senile plaques, with lower levels diffusing to CSF. Accordingly, studies have found a strong correlation between low Aβ42 in CSF and high numbers of plaques in the neocortex and hippocampus (Strozyk et al., 2003) or high retention of Pittsburgh Compound-B (PIB) in positron emission tomography (PET) scans that directly reflect plaque pathology in the living brain (Fagan et al., 2006; Forsberg et al., 2008). However, some studies have also found a marked reduction in CSF Aβ42 in disorders without Aβ plaques, such as Creutzfeldt–Jakob disease (CJD) (Otto et al., 2000), amyotrophic lateral sclerosis (Sjogren et al., 2002) and multiple system atrophy (Holmberg et al., 2003). These findings suggest that there may be other reasons for low CSF Aβ42 in addition to deposition of Aβ in plaques. Factors that may contribute to reduced Aβ42 levels, in addition to deposition in senile plaques, include formation of Aβ42 oligomers that escape ELISA detection (Stenh et al., 2005), association with other molecules that block access to epitopes recognized by detection antibodies, e.g., binding of Aβ42 to apolipoprotein E4 or other chaperone-like amyloid-binding proteins, such as β-trace protein (Kanekiyo et al., 2007), or cystatin C (Sastre et al., 2004), and sequestering of Aβ42 in the plasma membrane or intracellularly with lower levels diffusing to CSF (LaFerla et al., 2007). CSF levels of Aβ42, especially together with total tau (t-tau) can distinguish subjects with MCI who are likely to progress to AD with high sensitivity, specificity and predictive values, and may even be useful as markers for pre-clinical AD (Table 1).

CSF Aβ40 is unchanged or slightly increased in AD (Fukuyama et al., 2000; Hansson et al., 2007; Kanai et al., 1998; Mehta et al., 2000; Shoji et al., 1998). Consequently, a decrease in the ratio of Aβ42/Aβ40 (or increase in the ratio of Aβ40/Aβ42) in CSF has been found in AD in several papers (Fukuyama et al., 2000; Hansson et al., 2007; Kanai et al., 1998; Mehta et al., 2000; Shoji et al., 1998). This decrease in the ratio of Aβ42/Aβ40 seems more pronounced than the reduction of CSF Aβ42 alone (Hansson et al., 2007; Vigo-Pelfrey et al., 1993). Of note, some recent studies in individuals with genetically determined AD support that the ratio of Aβ42/Aβ40 may be more important to the neurobiology of AD than the absolute level of Aβ42 (Bentahir et al., 2006; Kumar-Singh et al., 2006).

Besides Aβ40 and Aβ42, the major products of concerted BACE1- and γ-secretase-mediated cleavages of APP (Fig. 2), CSF contains

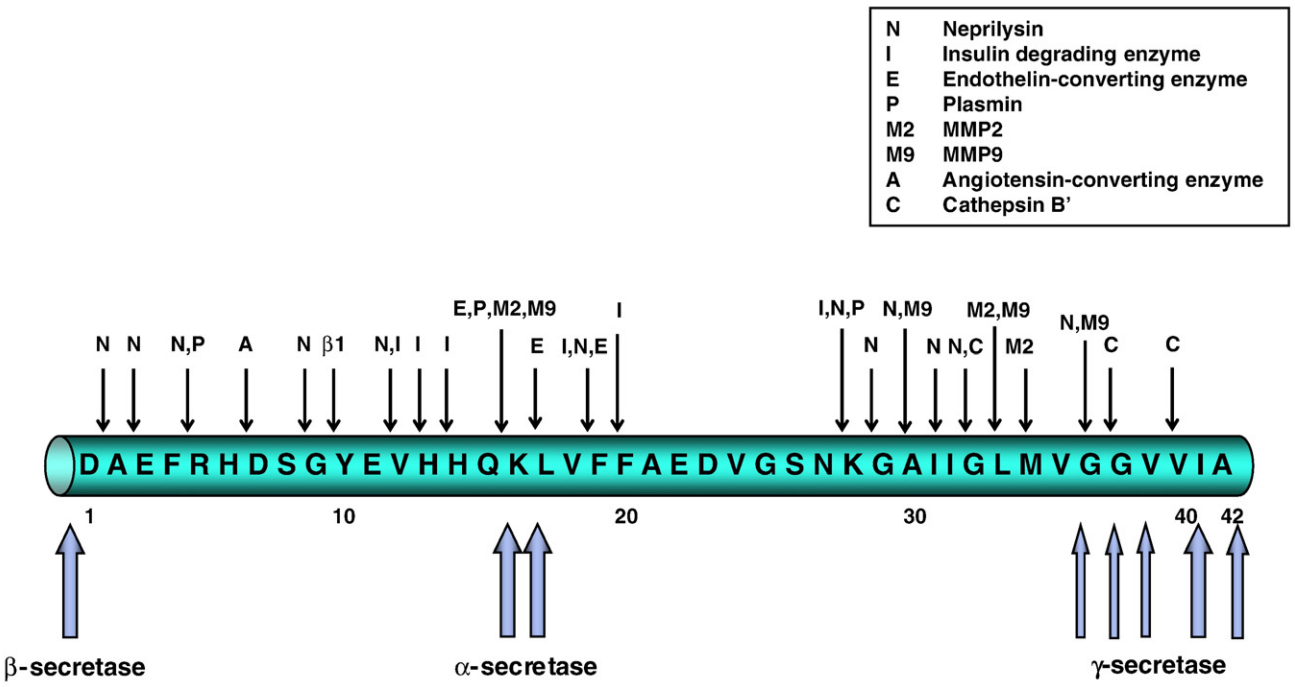

Fig. 4. Degradation of amyloid β (Aβ) by proteases. The 42 amino acid Aβ sequence is shown with the α-, β- and γ-secretase sites indicated. β′ indicates a second cleavage site of BACE1. The major Aβ-degrading enzymes (IDE = insulin-degrading enzyme; NEP = neprilysin; ECE = endothelin-converting enzyme; MMPs = matrix metalloproteinases; ACE = angiotensin-converting enzyme) are also represented. For a detailed review on their respective cleavage sites, see Andreasson et al. (2007).

several at least 20 truncated Aβ isoforms. The N- and C-terminal heterogeneity of Aβ peptides in part reflects the use of alternative cleavage sites by both BACE1 which can cleave either at Asp1 or at Glu11 and γ-secretase which can liberate Aβ terminating at residues 38, 40, 42 and 43 (Fig. 4). In addition several of the other Aβ isoforms detected in CSF likely arise due to partial degradation by the action of one or more Aβ degrading enzymes found in CSF. Using urea-based sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot, it is possible to separate several C-terminally truncated Aβ peptides in CSF, including Aβ37, Aβ38, Aβ39, Aβ40, and Aβ42 (Wiltfang et al., 2002). In AD, elevated CSF levels of both Aβ40 and Aβ38 are found, along with a reduction in Aβ42. Similar data have been obtained using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (Lewczuk et al., 2003; Sergeant et al., 2003). Other promising findings include those of different N-terminally truncated Aβ species present in protein extracts from AD brains (Sergeant et al., 2003). Some of these fragments are also detectable in human CSF and may be of diagnostic utility in early AD (Vanderstichele et al., 2005). A recent study identified a set of 18 different N- and C-terminally truncated Aβ peptides in CSF using immunoprecipitation–mass spectrometry(Portelius et al., 2006a). Their relative abundance pattern distinguished AD from controls with an accuracy of 86% (Portelius et al., 2006b). This technique has recently been optimized for large-scale studies by automation and the use of isotopically labelled internal standards that reduce the coefficients of variation for the different Aβ fragments to 5–15% (Portelius et al., 2007). Further studies on large patient and control series are now needed to determine the diagnostic potential of Aβ fragment signatures in CSF more precisely.

Aβ40 and Aβ42 in plasma

Many studies have examined plasma levels of Aβ in AD but the findings are contradictory (Table 2). Some groups report high concentrations in plasma of either Aβ42 or Aβ40 in AD, although with a broad overlap between patients and controls, whereas most groups find no change (Irizarry, 2004). Some studies have also reported high plasma Aβ42 in non-demented elderly people who later developed either progressive cognitive decline or AD (Mayeux et al., 2003; Pomara et al., 2005). Contrary to these data, one recent study found an association between high Aβ40, low Aβ42, and risk of dementia (van Oijen et al., 2006), a result that is in general agreement with the findings from other studies, finding a weak association between low plasma Aβ42/Aβ40 ratio and risk of future MCI or AD in a healthy, elderly population (Graff-Radford et al., 2007). Apart from disease-related factors, the opposing data may be due to analytical difficulties. The peptide is very hydrophobic and binds, not only to certain test tube walls, but also to several plasma proteins, including albumin (Kuo et al., 1999) and low-density lipoprotein receptor-related protein-1 (Sagare et al., 2007). Additionally, measurement of soluble Aβ has been achieved using assays that cannot identify the aggregation state of the species detected and may under detect Aβ oligomers (Stenh et al., 2005). Both plasma protein binding and oligomerization could mask Aβ epitopes, resulting in the measurement of only a fraction of Aβ. This possible confounder might differ between ELISA methods, which could explain some of the contradictory results. Moreover, the development of anti-Aβ oligomer-specific antibodies should obviate concerns about epitope masking due to Aβ self-association and may provide a useful system to measure Aβ oligomer levels in both CSF and plasma. Indeed a small number of preliminary studies suggest that measurement of Aβ oligomers will be of benefit (Georganopoulou et al., 2005; Pitschke et al., 1998). If this holds true in larger studies one would anticipate that combining measurement of disease-linked assembly forms (oligomers) of Aβ together with measurement of tau in CSF together with brain imaging will provide a highly specific and sensitive means of measuring both early and incipient AD. Indeed even in absence of structure-specific assays plasma Aβ might still be useful as a marker to identify and monitor biochemical effects of new amyloid-targeting drugs, a hypothesis that is supported by recent studies on γ-secretase inhibitors (Fleisher et al., 2008; Siemers et al., 2005, 2007, 2006) and immunotherapy (Relkin et al., 2008).

Table 2
Summary of studies on plasma Aβ in mild cognitive impairment, Alzheimer's disease and dementia.

Reference	Year	Setting	Cohort and outcome	Main finding – cross sectional	Main finding – longitudinal change	Comment
(Mayeux et al., 1999)	1999	Population-based study	Normal elderly - development of AD	High baseline Aβ42 and Aβ40 in incident AD	No change in incipient AD	Only baseline Aβ42 significant after statistical adjustments
(Mayeux et al., 2003)	2003	Population-based study	Normal elderly and MCI—development of AD	High baseline Aβ42 in incident AD	Longitudinal decline in Aβ42 in incipient AD	
(van Oijen et al., 2006)	2006	Population-based study	Normal elderly—development of AD	High baseline Aβ40 (but not Aβ42) in incident AD	NE	
(Graff-Radford et al., 2007)	2007	Cohort study, primary care	Normal elderly—development of MCI or AD	Low baseline Aβ42/Aβ40 ratio in incident MCI and AD	NE	
(Blasko et al., 2008)	2008	Community-based cohort study	Normal elderly—development of MCI or AD	No change in baseline Aβ42 in incident AD	Longitudinal in Aβ42 in incipient MCI and AD	
(Hansson et al., 2010)	2008	Cohort study, memory clinic	MCI—development of AD	No change in Aβ42 or Aβ40	NE	
(Lopez et al., 2008)	2008	Population-based study	Healthy elderly and MCI—development of AD	High baseline Aβ42 and Aβ40 in incident AD	Longitudinal increase in Aβ42 and Aβ40 in independently of cognitive change	
(Schupf et al., 2008)	2008	Cohort study of Medicare recipients	Normal elderly—development of AD	High baseline Aβ42 (but not Aβ40) in incident AD	Longitudinal decrease in Aβ42 (but not Aβ40) in incident AD	
(Sundelof et al., 2008)	2008	Population-based study	Normal elderly—development of AD	Low baseline Aβ40 (but not Aβ42) in incident AD at age 77.	No longitudinal change in Aβ42 or Aβ40 in incident AD	No change in Aβ40 or Aβ42 in prodromal AD at age 70
(Roher et al., 2009)	2009	Longitudinal case–control study	AD and non-demented controls	No change in Aβ42 or Aβ40	No disease-associated change over time	

The study had to include more than 100 subjects, and have a follow-up examination with cognitive outcome.
Abbreviations: AD = Alzheimer's disease; MCI = mild cognitive impairment; NE = not examined.

Human antibodies against Aβ-related proteins

Work in transgenic mouse models has suggested that antibodies directed at Aβ, generated by passive or active immunization, may help clear Aβ and reduce cognitive/mnemonic deficits (Bard et al., 2000; Schenk et al., 2000). Although active immunization does not so far appear viable in humans, owing to uncontrolled inflammatory responses following multiple administrations of the immunogen, it has generated ancillary interest in the possibility that humans may naturally develop antibodies to Aβ. However, some of these antibodies will be in pre-formed anti-Aβ antibody complexes and the variable results obtained in different studies may in part be explained due to use of assays that differ in their ability to detect anti-Aβ antibody complexes. Thus, disrupting anti-Aβ antibody complexes is essential in order to accurately measure total anti-Aβ antibody levels. A recent study employing such a strategy did indeed find significant differences in serum antibodies to Aβ between AD and aged-matched control subjects (Gustaw et al., 2008).

Whether such antibodies might be helpful, harmful, or neutral with respect to the development and progression of AD remains undetermined. Likewise, it is unclear what conditions induce formation of such antibodies, or how specific they are to AD. A plaque-killing assay to detect the presence of anti-Aβ antibodies revealed that approximately 50% of AD and 50% of control cases were positive (Xu and Gaskin, 1997). These findings are generally consistent with the report of Hyman et al. (2001) who found low but detectable anti-Aβ autoantibodies in just over 50% of all patients, and modest levels in under 5% of all patients. In CSF, however, significantly lower titers of anti-Aβ antibodies have been observed in AD compared to ND subjects using an ELISA (Dodel et al., 2002; Du et al., 2001, 2003). Recent data from Henkel and co-workers (2007) provide further support that IgG-Aβ complexes in CSF may be a protective factor against AD, but their potential as biomarkers is uncertain.

Biomarkers of Aβ-related mechanisms in drug development

CSF biomarkers may be valuable in clinical trials in at least four different ways: as diagnostic markers, for patient stratification, as safety markers and to detect and monitor biochemical drug effects (Table 3). The first generation of MCI clinical drug trials, such as the donepzil and vitamin E trials (Petersen et al., 2005), recruited unselected heterogeneous MCI cases, meaning, that probably around half of the cases did not have AD-type neurodegeneration. This may have seriously reduced the ability to identify potential efficacy of a drug candidate. There could be reduced costs and numbers of recruited subjects in future trials that are enriched and stratified for MCI subjects using clinically meaningful CSF biomarkers (Hansson et al., 2006).

Since AD is a disorder with a slow progression of symptoms, identification of a change in the slope of deterioration due to intervention with a disease-modifying drug candidate will require very large patient cohorts and treatment duration of several years. Small, short-term clinical trials may be valuable to verify a biochemical effect also in patients with AD, before the expensive and time-consuming step is taken to larger phase II or III clinical trials. A summary of biomarkers as surrogate measures for treatment effects on Aβ-related mechanisms is presented in Table 4.

Presently, it is uncertain how the Aβ1–42 concentration in CSF might respond to treatment with efficacious drugs that target pathways leading to Aβ, production, fibrillization and/or amyloidosis in man (Gilman et al., 2005; Siemers et al., 2006). Studies in transgenic mice, however, provide evidence that reduced CSF Aβ1–42 levels are to be expected for short-term treatment with inhibitors of γ-secretase (Lanz et al., 2003, 2004). Similar results have recently been seen in a phase IIa study of the Aβ clearance-enhancing compound PBT2 (Lannfelt et al., 2008). Based on longitudinal studies of conditions involving acute neuronal injury (Hesse et al., 2001; Zetterberg et al., 2006) and data from the interrupted phase IIa AN1792 trial (Gilman et al., 2005), t-tau should decrease towards normal levels if a treatment is successful in inhibiting the neurodegenerative process in AD. The same may be expected for p-tau, although there are still no studies backing this hypothesis. The usefulness of other Aβ-related biomarkers, e.g., BACE1 activity, as biomarkers for treatment efficacy remains to be investigated. Nevertheless, the low intra-individual variability of CSF tau proteins and Aβ42 in 6-month and 2-year studies of AD and MCI patients is an important prerequisite for the use of these biomarkers that may reflect the effects of disease-modifying AD therapies in clinical trials (Blennow et al., 2007; Zetterberg et al., 2007).

Besides the use of CSF biomarkers to identify and monitor the biochemical effects of a disease-modifying drug, they may also be valuable tools for safety monitoring in trials with drugs with potential serious side effects, such as immunotherapy. The phase IIa AN1792 trial was interrupted since 6% of cases developed meningoencephalitis (Orgogozo et al., 2003). In routine clinical practice, CSF analysis is the standard method to diagnose encephalitis. Typical findings are an increase in CSF mononuclear cells together with signs of blood–brain barrier damage and intrathecal immunoglobulin production (Table 5). CSF may thus be a valuable tool as safety measures in this type of trials.

Table 3
Potential use of cerebrospinal fluid biomarkers in clinical trials.

Application	Explanation	Time point for use
Clinical diagnosis	CSF biomarkers may be valuable in clinical trials on patients with early AD or MCI, to enrich the patient cohort with pure AD cases	Baseline evaluation of cases eligible for the trial
Stratification of cases	Cases with biomarker evidence of a disturbance in Aβ metabolism or deposition may show a better effect of anti-Aβ disease-modifying drug candidates	Baseline evaluation of cases eligible for the trial
Safety monitoring	Some cases treated with anti-Aβ drug candidates, such as Aβ immunotherapy, may have adverse events such as meningoencephalitis or vasogenic edema.	Baseline evaluation of cases and if a possible adverse event occurs
Theragnostics	CSF biomarkers may provide information that the drug has an effect on the biochemistry and pathogenic processes directly in patients with AD	Baseline evaluation and at time-points during the trial, including the last week of the trial

Limitations of animal models and cell-based research tools

Pre-clinical studies have benefited from the use of transgenic (Tg) rodents that express mutant forms of the human APP or PS genes. In these Tg mice, plaque deposition increases with time and defects in cognitive and synaptic function are observed (Spires and Hyman, 2005). Such genetically engineered mice are commonly used to evaluate if a drug candidate will reduce "Aβ burden," i.e., the number or extent of Aβ plaques in the brain. A pioneering study showed that immunization with Aβ1–42 in APP Tg mice reduced both Aβ burden and cognitive deficits (Lemere et al., 2006). However, the predictive value for translating data on drug effects from AD Tg mice to patients with AD seems to be low. In fact, there are more than 100 molecules that reduce Aβ plaque burden in these animal models, several of which have been found to lack any preventive effect or any clinical effect in treating patients with AD (Blennow et al., 2006). AD Tg mice

Table 4
CSF biomarkers to monitor the biochemical drug effect in clinical treatment trials in Alzheimer's disease.

Biomarker	Mechanism	Methodology	Direction of change	Comment
CNS Aβ42 Aβ42/Aβ40 ratio	CNS Aβ metabolism	ELISA (Andreasen et al., 1999a) Luminex (Olsson et al., 2005) ELISA (Hansson et al., 2007; Mehta et al., 2000)	Uncertain. May depend on both type of drug and time-point during treatment	CSF Aβ42 is the central biomarker to monitor Aβ accumulation in the CNS
Soluble APP isoforms (sAPPα and sAPPβ)	CNS APP metabolism	ELISA (Olsson et al., 2003) Meso-scale (Zetterberg et al., 2008)	Change depending on the type of drug	CSF sAPPβ may be valuable in clinical trials on, e.g., BACE1 inhibitors
BACE1 activity	CNS APP metabolism	Enzyme activity assays (Holsinger et al., 2004; Zetterberg et al., 2008; Zhong et al., 2007)	Change depending on the type of drug	CSF BACE1 activity may be a valuable biomarker for CNS APP metabolism in clinical trials of BACE1 inhibitors
T-tau	Intensity of neuronal degeneration	ELISA (Blennow et al., 1995) or Luminex system (Olsson et al., 2005)	Decrease in CSF tau with lower intensity of the neuronal degenerative process	CSF tau may be a valuable downstream biomarker to identify an effect on the neuronal degeneration
Phosphorylated tau protein (P-tau181 and P-tau231)	Tau phosphorylation	ELISA (Vanmechelen et al., 2000) or Luminex system (Olsson et al., 2005)	Decrease in CSF P-tau with lower tau phosphorylation	CSF P-tau may be a valuable downstream biomarker to identify an effect on the phosphorylation state of tau

have a huge over-expression of Aβ and develop plaques much faster than AD cases, and thus probably are much more responsive to anti-Aβ treatment than humans with sporadic AD.

APP transgenic mice over-expressing Aβ42 show learning and memory disruption, but do not show a significant loss of neurons, indicating that the transgenic rodents are incomplete models of neurodegenerative disease, and suggesting that Aβ1–42-induced memory deficits may involve more subtle neuronal alternations leading to synaptic defects in the absence of overt neuron loss (Jacobsen et al., 2006; Kamenetz et al., 2003). The extent to which these animal models recapitulate the AD phenotype depends on whether AD is primarily considered a disease of Aβ amyloid deposition that also manifests neurodegeneration, or whether it is primarily a neurodegenerative disease that secondarily manifests Aβ amyloid deposition (Swerdlow, 2007). These uncertainties call for caution when translating data from mice to man.

Perspectives

There is an extensive body of literature supporting the notion that analysis of Aβ42 in CSF together with other core feasible biomarkers, including t-tau and p-tau phosphorylated at either threonine 231 or 181, have reliably high diagnostic and predictive performance in identifying AD, even in the early symptomatic, predementia and clinical dementia stages (Hampel et al., 2004; Hansson et al., 2006; Zetterberg et al., 2003). Core feasible biological marker candidates of mechanisms related to AD pathology are in an ever-maturing development process and should inform regulatory guideline documents regarding study design and approval for novel compounds claiming disease modification. The more general use of CSF biomarkers in clinical practice may be justified, especially if some of the new disease-modifying treatments prove to have a positive clinical effect. The awareness of medical progress in the population will lead subjects with very mild or even only subtle subjective cognitive disturbances to seek medical advice, though in many cases the symptoms will be unrelated to AD neurodegeneration. Diagnostic tools, such as CSF biomarkers, may thus be needed to diagnose AD-spectrum disease at a very early stage in order to select appropriate candidates for treatment.

The authors are clearly aware that besides neurochemical candidate markers, a wide range of mostly computer-based analysis methods of structural and functional neuroimaging data hold great promise to substantially support early detection, prediction of cognitive decline and conversion to AD as well as mapping of effects of therapy on the brain (Hampel et al., 2008). These emerging in vivo-imaging tools, however, are often very expensive and not widely distributed or accessible for clinical use. Automated approaches are in the process of earlier testing. The rate of hippocampal atrophy assessed by labor-intensive manual MR-volumetry is currently the best MR-derived biomarker. However, other neuroimaging approaches show promise, including fully automated, observer- and a priori hypothesis-independent MR-

Table 5
CSF biomarkers for safety monitoring in clinical treatment trials in Alzheimer's disease.

Biomarker	Mechanism	Methodology	Direction of change	Comment
CSF cell count (mononuclear and polynuclear cells)	Inflammatory process in CNS	Microscopy (standard CSF cell count)	Increased number of mononuclear cells in inflammatory processes in the CNS	An increase in CSF mononuclear cells is an general indicator of CNS inflammation, such as encephalitis
CSF/serum albumin ratio	Blood–brain barrier (BBB) dysfunction/damage (Tibbling et al., 1977)	Immunonephelometry	Increased CSF/serum albumin ratio in cases with BBB damage	BBB damage is found in encephalitis and other processes affecting the brain capillaries and parenchyma (including neurodegenerative disorders)
Intrathecal IgG and IgM production	IgG (Tibbling et al., 1977) and IgM (Forsberg et al., 1984) index	Immunonephelometry	Increased IgG and/or IgM index	Intrathecal IgG/IgM production is a measure of CNS inflammation (including aseptic encephalitis) and/or humoral immune response,
	IgG (Blennow et al., 1994) and IgM (Sharief et al., 1990) oligoclonal bands in CSF	Electrophoretic techniques	Presence of IgG and/or IgM oligoclonal bands specifically in CSF	
Total tau (T-tau) protein	Neuronal damage	ELISA (Blennow et al., 1995) or Luminex system (Olsson et al., 2005)	Increase in acute neuronal damage (Hesse et al., 2000; Nylen et al., 2006a; Nylen et al., 2006b; Ost et al., 2006; Zetterberg et al., 2006)	CSF T-tau and NFL are sensitive biomarkers to identify acute or chronic processes with neuronal damage
Neurofilament light (NFL) protein		ELISA (Rosengren et al., 1996)		

based voxel- and deformation-based morphometry (VBM, DBM) (Teipel et al., 2004, 2007a), cortical thickness analysis (Lerch et al., 2005; Teipel et al., 2009),and region-of-interest analyses of the medial temporal lobe and the basal forebrain (Teipel et al., 2005). The application of machine learning algorithms to fMRI data (Mourao-Miranda et al., 2005) and structural and functional connectivity studies of altered neuronal fiber pathways organized in cognitive networks in the AD brain (Bokde et al., 2006; Teipel et al., 2007b) using diffusion tensor imaging, fMRI and PET, or even direct labeling of Aβ plaques with recently developed radioligands in molecular imaging yield particularly promising perspectives.

Combination and integration of multimodal imaging, genetic and neurochemical markers is still in its infancy; however, there are early studies combining CSF and MRI markers (Hampel et al., 2005) or CSF pattern and regional cerebral blood flow for added value (Haense et al., 2008; Hansson et al., 2009).

These complementary methods, among many others, need further evaluation in ongoing large-scale multi-center initiatives, such as ADNI. Presently, there are only a few studies in which the diagnostic accuracy and indication of the effects of new disease-modifying therapies of different biomarker candidates (CSF Aβ, t-tau, p-tau, MRI based region-of-interest measurement of hippocampal and whole brain atrophy, and 11C-PIB-PET) are progressing to an advanced stage of qualification as biomarkers with characteristic functions and directly compared. Moreover, this dynamically developing field requires additional data on added value, as well as cost-benefit analyses of individual and combinations of biomarkers. A reasonable 5-year perspective is that the utility of these biomarkers will be conclusively established and qualified in large-scale prospective and controlled multi-center trials, such as the US and European ADNI studies as well as in ongoing population-based prospective studies (Mueller et al., 2005). Most important, ongoing anti-amyloid drug development programs may demonstrate the utility of core feasible CSF biomarkers in early dose-finding studies, and in later proof-of-mechanism and concept studies. If so, it is reasonable to anticipate that such markers will eventually allow the selection of asymptomatic individuals at very high risk for later neurodegeneration who are therefore candidates for anti-amyloid therapy. It appears plausible that the biomarkers (as surrogate markers or markers of mechanisms of action) themselves will become the primary targets of therapy; that is, like in other areas of medicine (i.e., in oncology or in cardio-vascular diseases) drug candidates may be approved for to treat abnormal levels of the biomarker. In other words, CSF biomarkers of Aβ amyloid dysregulation may become true surrogate markers of AD neurodegeneration.

Acknowledgments

The authors wish to thank Ms. Barbara Asam, Mr. Alfred T. Welzel and Ms. Yvonne Hoessler for technical assistance in the preparation of the manuscript. Our colleagues also are thanked for their contributions to the work summarized here which has been supported in part by grants from the Science Foundation Ireland (SFI), the Health Research Board (Ireland), the Health Service Executive (HSE; Ireland), Trinity College Dublin, the Alzheimer Association, to H.H., NIH (P30 AG10124, UO1 AG24904), and the Marian S. Ware Alzheimer Program. J.Q.T. is the William Maul Measy-Truman G. Schnabel Jr. M.D. Professor of Geriatric Medicine and Gerontology. H.Z. and K.B. are supported by the Swedish Research Council, the Swedish Brain Power project and the Alzheimer's Association. The ADNI is a partnership of collaborators at the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, academic investigators, pharmaceutical companies, the US Food and Drug Administration, the NIH Foundation, the Alzheimer's Association, and the Institute for the Study of Aging all of whom contributed to the development of the ADNI.

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