# PENN Biomarker Core of the Alzheimer's Disease Neuroimaging Initiative

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**Abstract**

There is a pressing need to develop effective prevention and disease-modifying treatments for Alzheimer's disease (AD), a dreaded affliction whose incidence increases almost logarithmically with age starting at about 65 years. A key need in the field of AD research is the validation of imaging and biochemical biomarkers. Biomarker tests that are shown to reliably predict the disease before it is clinically expressed would permit testing of new therapeutics at the earliest time point possible in order to give the best chance for delaying the onset of dementia in these patients. In this review the current state of AD biochemical biomarker research is discussed. A new set of guidelines for the diagnosis of AD in the research setting places emphasis on the inclusion of selected imaging and biochemical biomarkers, in addition to neuropsychological behavioral testing. Importantly, the revised guidelines were developed to identify patients at the earliest stages prior to full-blown dementia as well as patients with the full spectrum of the disease. The Alzheimer's Disease Neuroimaging Initiative is a multicenter consortium study that includes as one of its primary goals the development of standardized neuroimaging and biochemical bio-marker methods for AD clinical trials, as well as using these to measure changes over time in mildly cognitively impaired patients who convert to AD as compared to the natural variability of these in control subjects and their further change over time in AD patients. Validation of the biomarker results by correlation analyses with neuropsychological and neurobehavioral test data is one of the primary outcomes of this study. This validation data will hopefully provide biomarker test performance needed for effective measurement of the efficacy of new treatment and prevention therapeutic agents.

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## Alzheimer's Disease

Alzheimer's disease (AD) is a progressive, neurodegenerative disease characterized by the loss of memory severe enough to affect a person's work, lifelong hobbies or social life. This and other symptoms vary widely. Other symptoms include confusion, trouble with organizing and expressing thoughts, misplacing things, getting lost in familiar places, and changes in personality and behavior. The most common form of AD, so-called sporadic AD, is a disease of the aging, with an almost logarithmically increased incidence with age starting at about 65 years. In 2000, there were 4.5 million people in the US
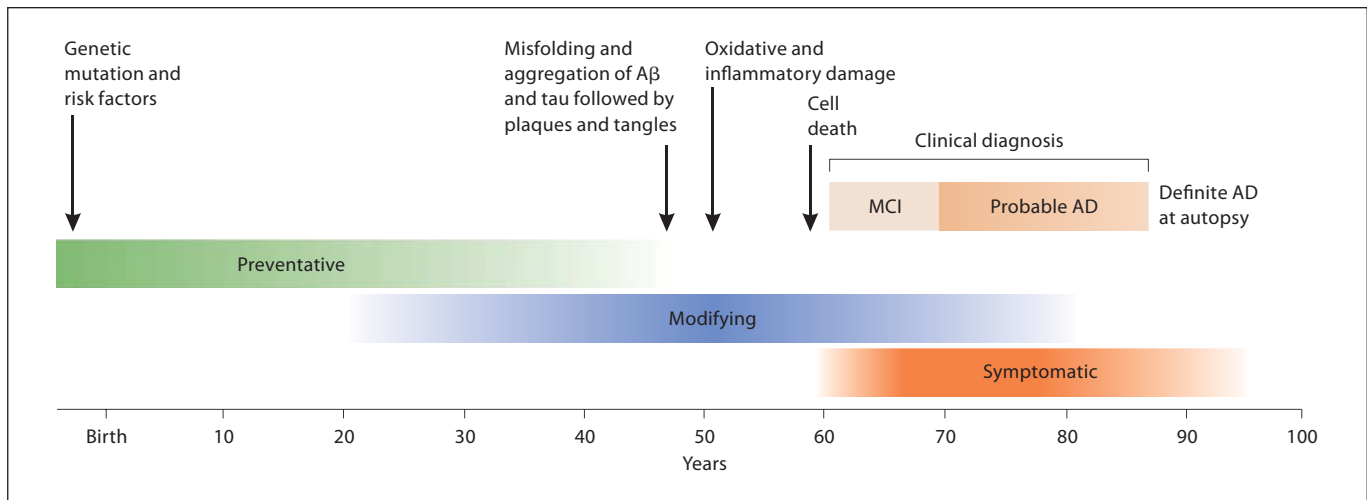
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**Fig. 1.** Hypothetical timeline for the onset and progression of sporadic as well as familial AD neurodegeneration and dementia. The green-, blue- and magenta-shaded bars indicate the time points at which preventative, disease-modifying and symptomatic interventions are likely to be most effective. From Shaw et al. [10], permission requested.

with AD and it was estimated that by the year 2050 there will be an almost 3-fold increase to 13.2 millions [1], a finding consistent with our aging population. A few therapeutics are currently available, but they only treat AD symptoms, and therefore we have a pressing need for developing prevention and disease-modifying treatment strategies for this devastating disease. It has been estimated that if such new strategies succeeded in delaying AD by 5 years, the number of affected individuals would be reduced by 50% over the next 50 years [2]. A major effort is underway by pharmaceutical companies to develop disease-modifying therapeutics based on the known pathophysiologic mechanisms of AD and a number of strategies for disease prevention are under investigation as well.

*Synopsis of AD Pathology and Mechanisms*

At the level of tissue pathology, the defining lesions of AD are neurofibrillary tangles and senile plaques formed, respectively, by neuronal accumulations of abnormal hyperphosphorylated tau filaments and extracellular deposits of amyloid β (Aβ) fibrils, mostly the 1–42 peptide (Aβ$_{1-42}$) which is the least soluble of the known Aβ peptides produced from amyloid precursor protein by the action of various peptidases. Both neuronal accumulations of tau filaments and extracellular deposits of Aβ are implicated in mechanisms of AD brain degeneration [3–5]. It is believed that the development of full-blown AD takes

place over 20–30 years of time (fig. 1) and a critical issue for the AD field is identification at the earliest time possible of individuals who go on to develop this disease so that any intervention would have its best chance of success.

The pathological aggregation of misfolded tau and Aβ that leads to their deposition in selectively vulnerable regions of the central nervous system is a mechanism shared by other neurodegenerative diseases such as Parkinson's disease (PD) and frontotemporal dementia (FTD). In PD the characteristic lesions, Lewy bodies (LB), are composed of abnormal α-synuclein filaments, whereas in FTD, AD-like fibrillary tau lesions are the characteristic finding. Another important and challenging characteristic of neurodegenerative diseases is their heterogeneity. For instance, in 15–30% of patients clinically diagnosed as having FTD, the underlying disorder is AD based on postmortem evaluation [6]. Furthermore, AD and PD commonly co-occur and the most common subtype of AD is the LB variant of AD, with more than 50% of AD patients showing LB in addition to senile plaques and neurofibrillary tangles [3, 5, 7–9].

*AD Diagnostic Biomarkers*

Driven partly by AD drug discovery research, AD is at the forefront of biomarker development for neurodegenerative diseases, and many current concepts about ideal biomarkers for these disorders have come from AD re-

search [for more detailed discussion and references, see 10]. AD biomarker investigations, based on the known mechanisms involved in this disease, have shown in studies involving several thousand AD patients and a variety of normal and diseased control groups that cerebrospinal fluid (CSF) concentrations of tau, including several species of phospho-tau, are elevated, whereas CSF concentrations of $A\beta_{1-42}$ are decreased in comparison to control subjects [11, 12]. Elevated levels of tau in CSF are thought to result via release from damaged and dying neurons, and depressed CSF levels of $A\beta_{1-42}$ are believed to result from large-scale accumulation of this least soluble of $A\beta$ peptides in insoluble plaques in the brain. The combination of increased CSF concentrations of tau and phospho-tau species and decreased concentrations of $A\beta_{1-42}$ are considered to be a pathological signature and diagnostic of AD. Recent studies have shown that this combination of biomarker changes may predict the conversion to AD in patients with a diagnosis of mild cognitive impairment (MCI) who later convert to AD [13].

Definitive diagnosis of AD requires autopsy evaluation of the brain. The diagnostic accuracy for making the diagnosis based on neuropsychological and neurobehavioral examinations is far from perfect, and this is especially the case given the problem of the heterogeneity often observed in neurodegenerative diseases like AD as described above. Well-validated AD biomarker tests are not only needed for early and improved diagnosis, but increasingly so for epidemiological screening, monitoring disease progression and response to treatment, enriching clinical trials for specific subsets of patients or at-risk individuals, and studying brain-behavior relationships [10].

However, not all AD biomarkers will be informative for each of these clinical and research applications, and some analytes that are suitable for use in clinical diagnosis might not be useful for monitoring responses of AD patients to therapeutic interventions. Accordingly, AD biomarkers will have different as well as overlapping applications, but, as initially proposed by the Working Group on Biological Markers of Alzheimer's Disease [14], ideal AD biomarkers should be: linked to fundamental features of AD neuropathology, validated in neuropathologically confirmed AD cases, able to detect AD early in its course and distinguish it from other dementias, non-invasive, simple to use, and inexpensive.

*AD Risk Factor Biomarkers*
Plasma homocysteine concentrations and *APOE* genotyping are examples of risk factor biomarkers rather than diagnostic analytes, and they are 2 of the most important studied AD risk factor biomarkers [15, 16]. In comparison to CSF $A\beta_{1-42}$, total tau and phosphorylated species of tau, plasma total homocysteine concentrations and *APOE* genotyping do not provide sufficient sensitivity or specificity for distinguishing AD from normal controls or other neurodegenerative disorders, so they cannot be characterized as diagnostic tests. Nevertheless, based on extensive clinical studies, both have been shown to be the most robust risk factor assays with significant predictive power for the development of dementia including AD. Thus, one such study showed that there was a 4.5-fold increase in relative risk for autopsy-confirmed AD in subjects in the top third (>14 μM) of the plasma homocysteine distribution after adjusting for other known risk factors, compared with the bottom 30% (<11 μM) [17]. A large-scale community study showed that increased total homocysteine plasma concentrations up to 11 years before diagnosis are associated with an increased risk for development of dementia [18].

Mutations in the APP, PSEN1 and PSEN2 genes account for virtually all autosomal dominant inherited early-onset forms of familial AD (FAD), but FAD represents <5% of all AD cases [3–5]. In contrast to these autosomal dominant FAD genes, the APOE genotype affects risk for AD, with *APOE4* increasing risk and *APOE2* decreasing risk relative to *APOE3*. The mechanism for this contribution to the onset or progression of AD in a dose-dependent manner is not precisely known, although a number of studies strongly suggest that the $A\beta$ chaperoning functions of *APOE* influence whether and when $A\beta$ aggregates. Recently, it was shown that the rate of conversion to AD by individuals with MCI is significantly greater in *APOE4*-positive subjects [19]. Thus, predicting which patients are at greatest risk for conversion to AD is aided by *APOE* genotyping, but this information contributes little towards diagnosis of AD in individual patients. However, the assessment of biomarkers that are risk factors for AD is useful in diverse types of clinical investigations, including clinical trials of new AD treatments. For example, these analytes permit balancing study groups for known risk factors. Furthermore, when taken together with diagnostic biomarkers such as CSF $A\beta_{1-42}$ and tau, plasma homocysteine measurements and *APOE* genotyping have the potential to further improve the diagnosis as part of a panel of AD biomarkers.

*Proposed New Framework for AD Diagnosis*
The recognition of the importance of the development and validation of imaging and chemical biomarkers,

some of which may detect AD accurately before dementia occurs, was recently emphasized in a proposed new guideline for the diagnosis of AD at a preclinical stage of the disease that would supplant the well-established but now aged National Institute of Neurological Disorders and Stroke-Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) working group criteria [15]. Thus, the revised NINCDS-ADRDA criteria were developed to capture both the earliest stages, before full-blown dementia, as well as the full spectrum of the disease [20]. The new approach makes use of ongoing experience that defines a precursor to AD, MCI, in which the patient is not demented but suffers from memory disorder. Many, but not all, MCI patients progress to AD such that ~45% of individuals with MCI will convert to AD within 5 years [3–5]. The new proposed framework for the diagnosis of AD will hopefully encourage as rigorous as possible testing of the new imaging and chemical biomarkers and could lead to improved identification of patients for new treatment trials, and therefore more accurate and efficient assessment of new therapeutics.

## Alzheimer's Disease Neuroimaging Initiative

Thus, a major need in clinical studies of AD is for accurate characterization of the predictive value of AD biomarkers and longitudinal biomarker changes in patients with a clinical diagnosis of MCI who progress to AD. For this purpose, a longitudinal AD study was designed to refine and validate the biomarker methods that have shown promise for early detection of AD. This study, the Alzheimer's Disease Neuroimaging Initiative (ADNI), includes 3 cohorts of study subjects: cognitively normal control subjects, patients with a diagnosis of MCI and a third cohort consisting of AD patients.

The ADNI is a multicenter consortium study funded by the National Institutes of Health, companies and foundations. I co-lead this together with John Trojanowski and we enjoy the collaboration of Virginia M.-Y. Lee in this endeavor and the continued input of Chris Clark who leads the University of Pennsylvania (PENN) ADNI clinical site. The goals of ADNI include the development of standardized neuroimaging and chemical biomarker methods for AD clinical trials, determination of optimal methods for acquiring and processing brain images, validation of AD neuroimaging and chemical biomarker results by correlating them with neuropsychological and neurobehavioral test data from the ADNI, and provision of a database of all ADNI findings that will be available to qualified scientific investigators for further data mining [10].

The ADNI has enrolled cognitively normal elderly control subjects, patients with AD and subjects with a diagnosis of MCI from 58 study sites in the US and Canada for a 3-year observational study. Recruitment of study subjects reached the halfway point in August 2006 and by June 2007, enrollment of 800 subjects was completed. All study subjects undergo periodic neuropsychological and neurobehavioral evaluations, neuroimaging studies, blood and urine sample collections, and more than 50% are providing CSF samples, enabling longitudinal studies of chemical biomarkers over the 3-year study observation period.

In order to implement the ADNI mission, the ADNI Biomarker Core at PENN collects and banks all biological samples (DNA, plasma, serum, urine and CSF) from all participating sites, and is conducting studies on selected AD biomarkers including APOE genotype, tau and phosphorylated tau species, A$\beta$, isoprostanes and homocysteine. Although these analytes were selected for study in the PENN Biomarker Core based on a consensus of AD biomarker experts [15], the Core will make banked ADNI samples available for studies of additional biomarkers by other investigators according to procedures outlined on the ADNI website.

In preparation for the chemical biomarker measurements on ADNI fluids considerable efforts by the PENN Biomarker Core have been made to validate these test procedures. For instance, in collaboration with 4 industrial and 2 academic laboratory partners, we have conducted systematic testing of all major variables that can affect the test results using the Luminex multiplex immunoassay platform we selected to use for ADNI CSF assays for A$\beta_{1-42}$, tau and tau phosphorylated in the 181 threonine position. In settings with approximately 50% prevalence of AD and/or MCI, more than 50 studies have demonstrated clinical sensitivity and specificity for these biomarkers greater than 80% each. However, using the same ELISA reagents, the mean biomarker concentration value for AD patients has varied approximately 2.5-fold, indicating a serious biomarker standardization problem. Our collaborative validation study is the first of its kind in the AD biomarker field and we hope this study will promote setting a standard for how other biomarkers in the neurodegenerative diseases field will be validated for use in clinical trials and, where applicable, as diagnostic tests in clinical practice.

This development of an Alzheimer's disease biofluid repository and biomarker core laboratory at PENN is

now poised for extensive collaboration with other academic centers, as well as pharmaceutical and diagnostic companies, to study and evaluate new and established biomarkers for the early detection, monitoring progression and assessment of effects of disease modification of AD.

## Acknowledgements

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