BIOMARKERS

Biomarkers of neurodegeneration for diagnosis and monitoring therapeutics

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Abstract | Rapid progress towards understanding the molecular underpinnings of neurodegenerative disorders such as Alzheimer's disease is revolutionizing drug discovery for these conditions. Furthermore, the development of models for these disorders is accelerating efforts to translate insights related to neurodegenerative mechanisms into disease-modifying therapies. However, there is an urgent need for biomarkers to diagnose neurodegenerative disorders early in their course, when therapy is likely to be most effective, and to monitor responses of patients to new therapies. As research related to this need is currently most advanced for Alzheimer's disease, this Review focuses on progress in the development and validation of biomarkers to improve the diagnosis and treatment of Alzheimer's disease and related disorders.

Biomarker
A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention.

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Alzheimer's disease (AD) and many other neurodegenerative disorders share common mechanisms that are linked to the pathological aggregation of misfolded proteins that accumulate as fibrillar amyloid deposits in selectively vulnerable regions of the central nervous system (for reviews, see REFS 1–4). The defining lesions of AD are neurofibrillary tangles (NFTs) and senile plaques formed by neuronal accumulations of abnormal tau filaments and extracellular deposits of Aβ fibrils, respectively, both of which are implicated in mechanisms of AD brain degeneration[2–4] (FIG. 1). On the other hand, Lewy bodies (LBs) composed of abnormal α-synuclein filaments are pathological signatures of Parkinson's disease (PD), and growing evidence implicates abnormal α-synuclein in the pathogenesis of PD (for reviews, see REFS 5–9). For these and other reasons the progressive conversion of normal soluble tau and Aβ fibrils in AD and α-synuclein in PD to form insoluble oligomers, protofibrils and fully formed amyloid fibrils is increasingly the focus of drug discovery to identify disease-modifying therapies for AD, PD and related neurodegenerative brain amyloidoses[4]. Moreover, transgenic animal models of these disorders enable proof-of-concept studies of compounds that target disease-specific amyloidogenic pathways, and many of these compounds are entering clinical trials[4].

This progress in neurodegenerative disease research notwithstanding, a major impediment to the conduct of cost-effective and informative clinical trials of potential disease-modifying therapies is the absence of robust biomarkers for the early diagnosis of these disorders, when therapy is likely to have the greatest impact, and for monitoring patient responses to new therapeutic interventions in clinical trials. A daunting challenge to overcoming this obstacle is the complexity of neurodegenerative diseases, a number of which overlap[2,7,9]. This overlap is exemplified by neurodegenerative tauopathies characterized by AD-like fibrillary tau lesions, many of which manifest clinically as frontotemporal dementia (FTD). As illustrated in FIG. 2, various mechanistically diverse neurodegenerative diseases could underlie the clinical manifestations of FTD. Indeed, in 15–30% of patients meeting clinical criteria for FTD, the underlying disorder is AD on post-mortem examination[10]. Furthermore, AD and PD commonly co-occur, and the most common subtype of AD is the LB variant of AD, while >50% of AD patients show LBs in addition to senile plaques and NFTs[2,4,7,9,11]. In addition, although PD is a neurodegenerative α-synucleinopathy that manifests principally as a movement disorder, PD with dementia is common; when dementia precedes Parkinsonism and there are abundant cortical as well as subcortical LBs, the disorder is designated dementia with LBs[11]. FIGURE 3 illustrates the complexity of neurodegenerative movement disorders and the overlap of PD and AD.

So, there is a major need for biomarkers that facilitate the reliable distinction of different forms of dementia and movement disorders from one another for optimal treatment and management. Although the complexity highlighted above is a considerable challenge for the development of informative neurodegenerative disease

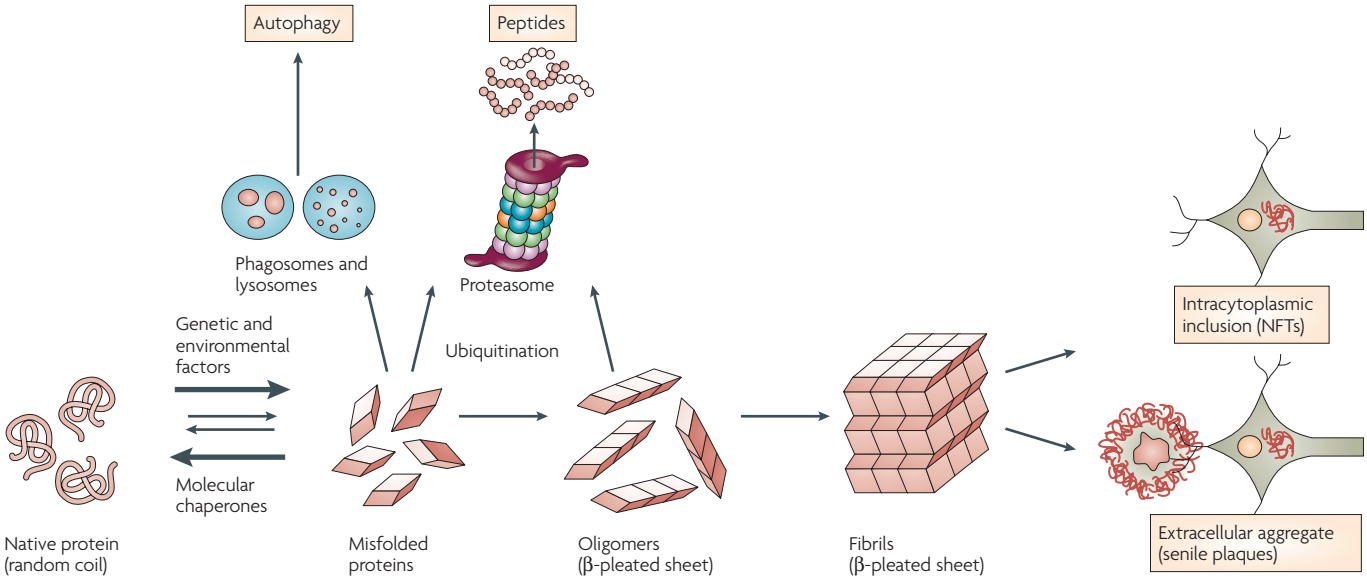

Figure 1 | **Proposed mechanisms underlying Alzheimer's disease.** Shown here is a model of protein misfolding and fibrillization, which leads to the deposition of aggregated tau filaments in neurons and fibrillar Aβ in the extracellular space of the brain afflicted with Alzheimer's disease (AD). Genetic and environmental factors can accelerate this process, whereas properly functioning cellular quality-control systems (molecular chaperones, ubiquitin proteasome system, phagosome and lysosome system) limit the accumulation of misfolded proteins. Ideal AD biomarkers should be linked to the mechanisms of neurodegeneration in AD. NFTs, neurofibrillary tangles.

biomarkers, there is growing optimism for efforts aimed at this goal, based to a large extent on progress in AD biomarker research, which is therefore the focus of this Review.

Overview of AD mechanisms

Aβ hypothesis. As reviewed elsewhere[3,4], the Aβ-centric focus of most AD drug discovery reflects the remarkable progress in understanding the role of Aβ fibrils in the pathogenesis of AD. This has culminated in the Aβ-cascade hypothesis of AD, which predicts that increased production, aggregation and accumulation of Aβ fibrils leads to senile plaques, neurotoxicity and the clinical manifestations of AD. Accordingly, most drugs in development for AD target Aβ amyloidosis by inhibiting or reducing the production of amyloidogenic Aβ peptides, or by promoting the clearance of Aβ oligomers and other Aβ aggregates[3,4]. FIGURE 4a illustrates points in the Aβ-metabolic pathways that could be targeted for therapeutic intervention to prevent conversion of mild cognitive impairment (MCI), a prodromal form of AD, to fully developed AD, or to ameliorate fully developed AD. Inhibitors of γ- and β-secretases that generate Aβ, passive and active Aβ vaccines, metal-binding drugs, statins, non-steroidal anti-inflammatory drugs and glycosaminoglycan mimetics are among the classes of compounds targeting Aβ-mediated neurodegeneration in AD that are in various stages of development now[4]. It would be highly desirable to have biomarkers that specifically reflect the effects of each of these mechanistically distinct interventions (summarized in FIG. 4) for use as surrogate markers in clinical trials of candidate compounds, and then to guide the optimal use of those drugs that become approved for the routine treatment of patients with AD.

Tau hypothesis. The tau hypothesis of AD neurodegeneration emerged from insights into the pathobiology of NFTs and the normal biology of tau[4]. For example, normal tau binds to and stabilizes microtubules that are essential for axonal transport, and the phosphorylation of tau negatively regulates the binding of all six normal brain tau isoforms to microtubules. Furthermore, as the subunits of paired helical filaments (PHFs) that form AD NFTs are abnormally phosphorylated forms of tau (PHFtau), it is not surprising that PHFtau is incapable of binding to and stabilizing microtubules, although this loss-of-function defect is reversed by enzymatic dephosphorylation of PHFtau[4]. On the basis of this and other information, the tau hypothesis of AD neurodegeneration predicts that the conversion of normal tau into functionally impaired PHFtau destabilizes microtubules, thereby disrupting microtubule-based axonal transport, which compromises the viability of affected neurons and leads to the onset and/or progression of AD (FIG. 4b). The discoveries of *tau* gene mutations that are pathogenic for hereditary FTD with Parkinsonism linked to chromosome 17 (FTDP-17) provided support for this hypothesis, as FTDP-17 is characterized by tau pathologies in the absence of senile plaques or other disease-specific lesions[12]. Indeed, FTDP-17 mutations result in losses of tau functions (that is, a loss of microtubule binding) and/or gains of toxic properties (that is, increased amyloidogenicity) and overexpression of wild-type and/or mutant tau in worms, flies and mice models are key features of tauopathies[13]. As illustrated in FIG. 4b, tau pathologies have become a focus for discovering

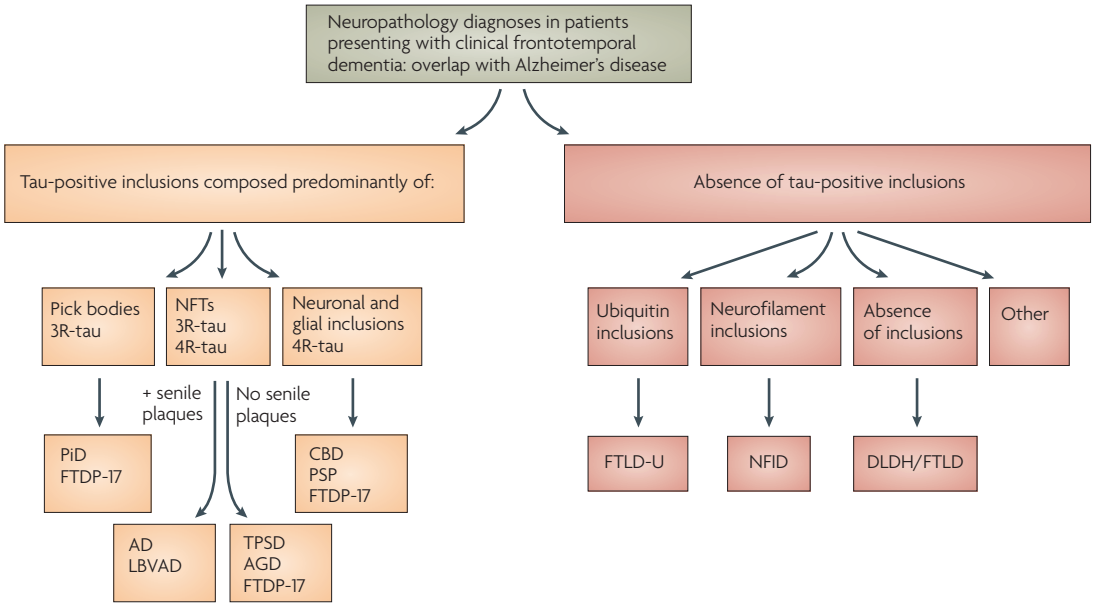

Figure 2 | **An algorithm for the neuropathological diagnosis of patients with clinical frontotemporal dementia.** Note that Alzheimer's disease (AD) is an underlying cause of frontotemporal dementia (FTD). Although most FTDs are tauopathies or associated with ubiquitin-positive, and tau- and α-synuclein-negative inclusions, the neuropathology of other FTDs is heterogeneous and the clinical manifestations of FTDs do not indicate the underlying neuropathology. Therefore more informative biomarkers are needed to distinguish AD from the other disorders shown here and in FIG. 3. Note: some of the disorders listed here and in FIG. 3 are double or triple brain amyloidoses because inclusions formed by multiple amyloidogenic proteins (for example, senile plaques formed by fibrillar Aβ, neurofibrillary tangles (NFTs) formed by phosphorylated forms of tau, and Lewy bodies formed by α-synuclein filaments) occur in these diseases. 3R-tau, tau isoforms with three microtubule-binding repeats; 4R-tau, tau isoforms with four microtubule-binding repeats; AGD, agyrophilic grain disease; CBD, corticobasal degeneration; DLDH, dementia lacking distinctive histopathology; FTDP-17, FTD with Parkinsonism linked to chromosome 17; FTLD, frontotemporal lobar degeneration, an alternative term for DLDH; FTLD-U, FTD with ubiquitin-positive but tau- and α-synuclein-negative inclusions; LBVAD, Lewy body variant of AD; NFID, neuronal intermediate filament disease; PiD, Pick's disease; PSP, progressive supranuclear palsy; TPSD, tangle predominant senile dementia.

disease-modifying therapies for AD and related tauopathies, and a number of compelling targets for tau-centered drug discovery are emerging[4,14].

For example, microtubule-stabilizing drugs might have potential therapeutic benefit by offsetting the loss of tau function owing to its sequestration of NFTs and/or its excessive phosphorylation[15], and these drugs might also ameliorate Aβ neurotoxicity in AD[16,17]. Furthermore, high-throughput screening is being used to identify drugs that block or reverse the fibrillization and aggregation of tau[18–20]. Preliminary high-throughput screening studies that target the inhibition of tau phosphorylation also seem promising, and proof-of-concept studies using LiCl to ameliorate tau pathology by inhibiting glycogen synthase kinase-3 (GSK3) in mouse tauopathy models suggest that this is a fruitful avenue for drug discovery[21]. Similar to microtubule-stabilizing drugs, GSK3 inhibitors might ameliorate Aβ and tau amyloid pathologies in AD[22]. Last, as discussed above for therapies that target Aβ, it would also be desirable to have biomarkers that specifically reflect the effects of candidate drugs that target different steps in tau-mediated neurodegeneration for use as surrogate markers in clinical trials and for treating MCI and AD patients with new disease-modifying interventions once they are approved.

Biomarker development: AD as an example

Driven in part 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 research[23,24]. With respect to the target population for AD biomarkers, this includes patients affected by either familial or sporadic AD; there is also growing interest in identifying markers of prodromal AD (that is, MCI), as well as assays that are predictive of AD years before its onset. These concepts have emerged from genetic studies that enable the identification of asymptomatic individuals with pathogenic mutations in the Aβ precursor protein (APP), presenilin 1 (*PSEN1*) and 2 (*PSEN2*) genes that cause autosomal dominantly inherited familial AD (FAD), and the recognition that individuals with MCI have an increased risk for developing AD within 3–5 years, such that ~45% of individuals with MCI will convert to AD within 5 years[2–4]. Indeed, there is growing evidence that the neurodegenerative pathways culminating in AD might be activated years before dementia becomes overt, as illustrated in FIG. 5. In fact, for FAD, one can argue that these pathways are activated at conception, but they require 20–30 years to result in the development of overt AD pathology.

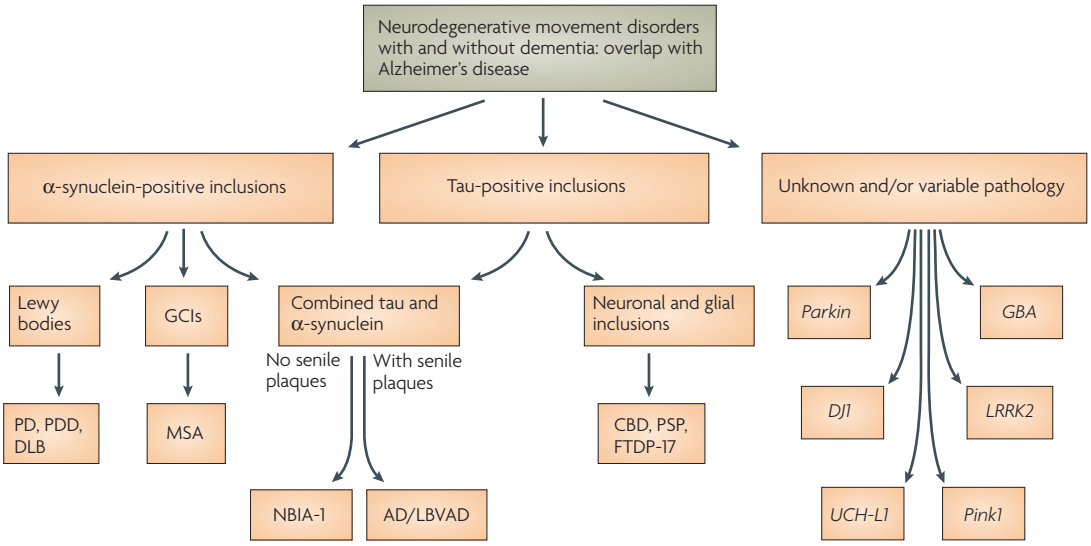

Figure 3 | **An algorithm for the neuropathological diagnosis of sporadic or familial neurodegenerative movement disorders including Parkinson's disease, Parkinson's disease with dementia and dementia with Lewy bodies.** The neuropathology in most patients with L-DOPA-responsive Parkinsonism reveals α-synuclein-positive Lewy bodies and Lewy neurites. Notably, there is clinical overlap between Alzheimer's disease (AD), Parkinson's disease (PD), PD with dementia (PDD) and dementia with Lewy bodies (DLBs), whereas the most common subtype of AD is the Lewy body variant of AD (LBVAD). CBD, corticobasal degeneration; FTDP-17, FTD with Parkinsonism linked to chromosome 17; *GBA*, glucosidase-β acid; GCI, glial cytoplasmic inclusions; *LRRK2*, leucine-rich repeat kinase 2; MSA, multiple system atrophy; NBIA-1, neurodegeneration with brain iron accumulation 1; PSP, progressive supranuclear palsy; *UCH-L1*, ubiquitin carboxy terminal hydrolase.

Sensitivity
In this case, a sensitivity of 100% indicates that a diagnostic test identifies all patients with AD.

Specificity
In this case, a test with 100% specificity identifies all individuals free of AD.

Prior probability
The background prevalence of the disease in the population tested.

Positive predictive value
The positive predictive value of an AD biomarker refers to the percentage of people who are positive for the biomarker and have definite AD at autopsy. A positive predictive value of 100% indicates that all patients with a positive test have the disease.

Negative predictive value
The percentage of people with a negative test who, at autopsy, prove not to have the disease. A negative predictive value of 100% indicates that the test completely rules out the possibility that the individual has the disease when the test is performed.

AD biomarkers could therefore have multiple uses, such as identifying those at greatest risk of developing AD, confirming the diagnosis of AD, epidemiological screening, predictive testing, monitoring disease progression and response to treatment, enriching clinical trials for specific subsets of patients or at-risk individuals, and studying brain–behaviour relationships[23–30]. 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[23], 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.

All AD biomarkers require evaluation of their sensitivity, specificity, prior probability, positive predictive value and negative predictive value (Supplementary information S1 (table)). For a biomarker to be useful in the diagnosis of AD, it should have a sensitivity and specificity of >85%, and a positive predictive value of >80%.

On the basis of extensive studies to date on potential AD biomarkers, it is likely that a combination of biomarkers will provide greater diagnostic accuracy than any single analyte[23–30], and the simultaneous evaluation of multiple biomarkers should use the measures outlined above. Although the initial Working Group on Biological Markers of Alzheimer's Disease recommended the validation of AD biomarkers by at least two independent studies from qualified investigators in studies published in peer-reviewed journals[23,24], in reality, multiple independent studies are needed, including multi-site analyses to define standard operating procedures for the use of diagnostic AD biomarker tests in clinical settings as well as in research laboratories. Last, it would useful for clinical trials of new AD therapies if the biomarker reflected the beneficial effect of the disease-modifying therapy[23–30].

However, the quest to find the ideal AD biomarker or panel of ideal AD biomarkers has not yet culminated in complete success, and finding the 'pregnancy test' equivalent for diagnosing AD at its earliest stages (that is, MCI) or before it manifests overtly is challenging. Nonetheless, as summarized below and in TABLE 1 (which lists additional emerging AD biomarkers other than those discussed below, on the basis of prior consensus reports[23,24] and current literature), there are promising and emerging candidate assays that, with further research, could become part of a panel of informative AD biomarkers.

Promising candidate AD biomarkers

Several candidate AD biomarkers have emerged during the past decade. As reviewed by a second AD biomarker work group[24] and other investigators[25–30], isoprostanes, tau, Aβ, sulphatides and homocysteine are among the most promising AD biomarker candidates, but there are other potential AD biomarkers, such as Aβ precursor proteins, apolipoprotein E (APOE), 8-hydroxy-2′-deoxyguanosine, α1-antichymotrypsin, interleukin-6 (IL-6), IL-6-receptor-complex proteins, C-reactive

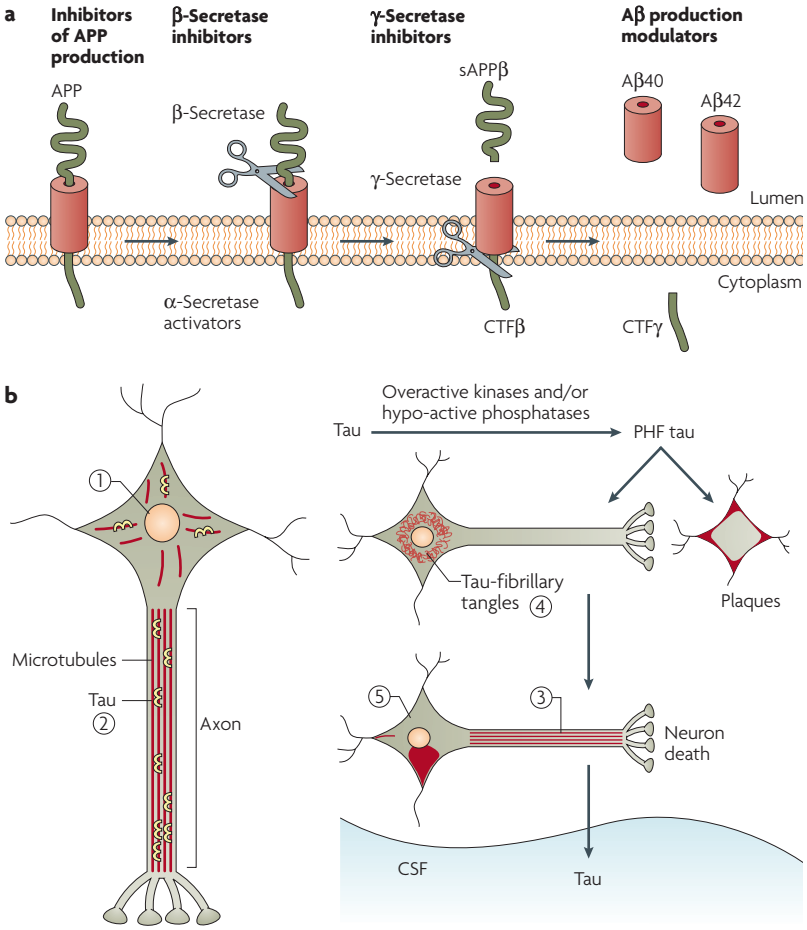

Figure 4 | **Possible targets for therapeutic intervention in Aβ and tau metabolic pathways. a** | Aβ precursor proteins (APP) are cleaved by β-secretase (also known as β-site APP-cleaving enzyme; BACE) to yield secreted APPβ (sAPPβ) and C-terminal fragment-β (CTFβ). CTFβ is a then cleaved by γ-secretase within membranes to yield CTFγ and Aβ fragments (red). An alternative cleavage by α-secretase (not shown) cuts within Aβ and precludes Aβ production. Potential opportunities for therapeutic intervention are shown in bold. **b** | The misfolding, fibrillization and sequestration of tau into filamentous phosphorylated forms of tau (PHF tau) inclusions (for example, neurofibrillary tangles and dystrophic tau neurites) is schematically depicted here. As described in greater detail in the text, this compromises the survival of neurons by depleting levels of functional tau below a critical level, which results in the depolymerization of microtubules and disruption of axonal transport. The release of tau from dying neurons might account for elevated levels of tau in cerebrospinal fluid (CSF), which is a biomarker of Alzheimer's disease. The numbers indicate tau-focused interventions to: 1) suppress levels of mutant tau or correct imbalances in the 3R:4R tau ratio, 2) increase elimination of pathological tau or reverse tau hyperphosphorylation, 3) offset sequestered tau by stabilizing microtubules to maintain normal axonal transport, 4) prevent or reverse tau oligomerization, fibrillization and aggregation, 5) provide neuroprotection for affected neurons. Alzheimer's disease biomarkers are needed that reflect the ameliorative effects of the Aβ- and tau-focused therapies indicated here.

protein and C1q protein (TABLE 1). Here, we summarize the current status of AD biomarkers according to the criteria outlined above, with a focus on those that were selected for in-depth analysis in the Alzheimer's Disease Neuroimaging Initiative (ADNI), a recently launched public–private research programme to define and validate informative neuroimaging and chemical biomarkers of AD and the transition from MCI to early AD[31] (BOX 1).

ELISA
Enzyme-linked immunosorbant assay. An immunochemical technique using antibodies to detect and quantify the presence of an antigen in a sample.

CSF tau and Aβ. Tau and Aβ are readily measured in cerebrospinal fluid (CSF) by ELISA, and they are the most extensively studied AD biomarkers, as thousands of patients with AD, as well as various normal and diseased control subjects, have been studied[23–30]. As discussed above, tau and Aβ are linked to the pathology of AD as well as to mechanisms of AD neurodegeneration. Measures of total tau as well as species of phospho-tau detected by antibodies specific for tau phosphorylated at Thr181, Ser199 or Thr231, in addition to Aβ1–42 (rather than Aβ1–40 or total Aβ), in CSF correlate best with a diagnosis of AD[24–26]. Total tau is two- to three-fold higher in CSF of patients with AD compared with normal controls. The release of tau (including species of phospho-tau) from degenerating neurons harbouring NFTs and dystrophic neurites in AD is thought to account for the increase in CSF levels of these proteins. So, the effects of therapies that ameliorate tau-mediated neurodegeneration and the further accumulation of species of pathological tau could be reflected in CSF tau biomarker assays. On the other hand, the massive accumulation of Aβ1–42 in pathological deposits in the AD brain is thought to result in the ~40% reduction in CSF Aβ1–42 levels in CSF of AD versus normal controls. Accordingly, the effects of therapies that ameliorate Aβ-mediated neurodegeneration and the accumulation of pathological species of Aβ could be reflected in assays for CSF Aβ levels. Further studies are needed to confirm the utility of these biomarkers for the diagnosis of MCI, but recent reports are promising[27–30]. Indeed, the combination of total tau levels and the Aβ1–42/phospho-tau (Thr181) ratio predicted progression to AD with a sensitivity and specificity of 95% and 87%, respectively, in one recent study of MCI subjects followed for 4–6 years[28]. Given the focus of AD drug discovery efforts on targets in tau- and Aβ-mediated neurodegeneration pathways, CSF tau and Aβ could become surrogate markers for the response of patients with AD to the novel therapies that are likely to emerge from these efforts.

CSF, plasma and urine isoprostanes. Oxidative damage is implicated in the pathogenesis of AD, and specific isoprostanes (for example, 8,12-iso-iPF$_{2\alpha}$-VI) produced by lipid peroxidation are elevated in urine, blood and CSF of patients with AD[4,24,27,29,30]. These values correlate with memory impairments, CSF tau levels and the number of APOE4 alleles[24,27,29,30], which suggests that 8,12-iso-iPF$_{2\alpha}$-VI is a useful AD biomarker. Isoprostanes are measured in CSF, blood, urine and postmortem brain using high-performance liquid chromatography/tandem mass spectrometry with atmospheric pressure chemical ionization. Additional studies are needed to validate this analytical methodology and to extend these findings to larger cohorts of patients with AD or MCI. Studies are also needed to determine whether 8,12-iso-iPF$_{2\alpha}$-VI will be informative for monitoring the response of patients with AD to new therapies in clinical trials of drugs that target tau and Aβ neurodegenerative pathways as well as those that reduce oxidative damage.

APOE4
Apolipoprotein E4 allele. The susceptibility gene variant that is the most robust genetic risk factor for common, late-onset AD.

Autosomal dominant
In this context, autosomal dominant refers to mutations (that is, changes in a gene) that are sufficient to cause disease when a single copy of the gene is affected by the mutation, and it is inherited by an individual who will develop the disease if he/she lives to the age of onset of the disease.

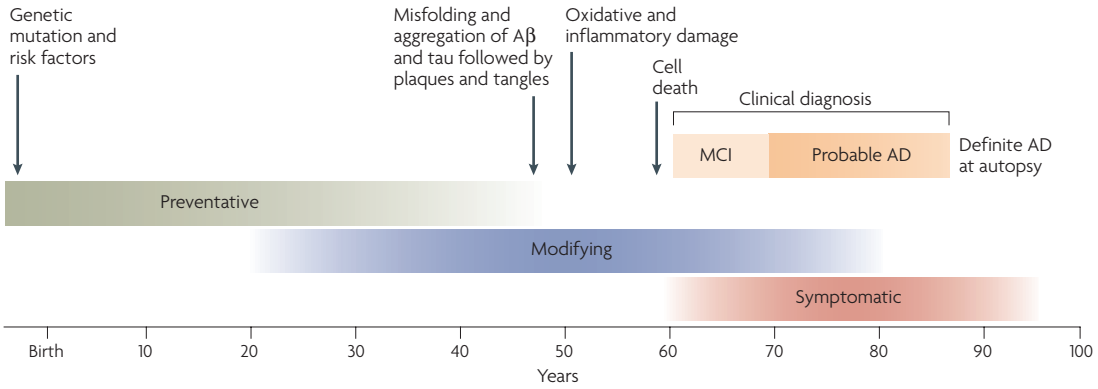

Figure 5 | **Hypothetical timeline for the onset and progression of sporadic as well as familial AD neurodegeneration and dementia.** There are few predictive biomarkers for Alzheimer's disease (AD) except genetic mutations that are pathogenic for familial AD, which could be measured from conception onwards, but the emphasis in this Review is on promising biomarkers for AD identified in consensus conferences and reviews[23–30], and especially those pursued in the Alzheimer's Disease Neuroimaging Initiative[31]. 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. AD biomarkers are needed to accelerate efforts to test the efficacy of preventative and disease-modifying therapies for AD. MCI, mild cognitive impairment.

Plasma total homocysteine and* APOE *genotyping. Plasma total homocysteine concentrations and *APOE* genotyping are examples of risk factor biomarkers rather than diagnostic analytes, and they are two of the most studied risk factor biomarkers for AD[24,30]. In comparison to CSF Aβ1–42, 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. However, on the basis of extensive clinical studies of a large number of patients with AD and appropriate control subjects, they have both been shown to be among the most robust risk factor assays with significant predictive power for developing dementia including AD. For example, in a study that included autopsy-proven cases of AD versus control subjects, total plasma homocysteine concentrations in the top third (≥14 μM) of the serum homocysteine distribution were associated with a 4.5-fold increase in relative risk for confirmed AD, adjusted for other known risk factors such as age, smoking and presence of the *APOE4* allele, compared with the bottom 30% (≤11 μM)[32]. 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[33].

Mutations in the *APP*, *PSEN1* and *PSEN2* genes account for virtually all autosomal dominant inherited early onset forms of FAD, but it is important to note that FAD represents a small percentage (<5%) of all AD cases[2–4]. 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*. It is not precisely known how *APOE* genotype contributes to the onset or progression of AD in a dose-dependent manner, although a number of studies strongly suggest that the Aβ chaperoning functions of APOE influence whether and when Aβ aggregates. In a study of several populations of patients with a clinical diagnosis of AD, 19–36% of the patients with AD and 10–16% of normal controls were *APOE4* positive and 40% of autopsy proven AD were *APOE4* positive[34], but not all individuals homozygous for the APOE4 allele will develop AD[2–4]. More recently, data has shown that the rate of conversion to AD by individuals with MCI is significantly greater in *APOE4*-positive

Table 1 | **Other potential Alzheimer's disease biomarkers**

Analyte	Biofluid	References
Aβ antibodies	Serum, plasma, CSF	48,49
α-Antichymotrypsin	Blood, CSF	50–54
Amyloid precursor protein (APP)	CSF	55–58
APP isoform ratio in platelets	Platelets	59–61
β-Secretase (also known as BACE)	Platelets	62
CD59	Serum, plasma, CSF	63,64
C-reactive protein	Serum, plasma, CSF	65,66
Clq	Serum, plasma, CSF	67,68
8-hydroxy-deoxyguanine	CSF, plasma, urine	69,70
Glutamine synthetase	Serum, CSF	71,72
Glial fibrillary acidic protein (GFAP) and antibodies to GFAP	CSF	73–76
Interleukin-6-receptor complex	Serum, CSF	77
Kallikrein	CSF, plasma	78
Melanotransferrin	Serum, CSF	79–81
Neurofilament proteins	CSF	82–84
Nitrotyrosine	CSF	85,86
Oxysterols	Plasma, CSF	87
Sulphatides	CSF	88,89
Synaptic markers	CSF	90
S100β	Blood, CSF	91,92

CSF, cerebrospinal fluid.

Box 1 | **The Alzheimer's Disease Neuroimaging Initiative**

Funded by the National Institutes of Health (NIH), companies and foundations, the goals of the Alzheimer's Disease Neuroimaging Initiative (ADNI) are:

- Develop standardized neuroimaging and biomarker methods for Alzheimer's disease (AD) clinical trials.
- Determine optimum methods for acquiring and processing brain images.
- Validate AD neuroimaging and biomarker findings by correlating them with neuropsychological and behavioural test data from the ADNI.
- Provide a database for all ADNI findings that will be available to qualified scientific investigators for further data mining.

ADNI is enrolling 200 cognitively normal elderly controls, 200 patients with AD and 400 subjects with MCI at ~60 clinical sites throughout the United States and Canada for a 3-year observational study. All subjects undergo periodic neuroimaging studies, blood and urine samples are collected from all subjects, and cerebrospinal fluid (CSF) is obtained from ~60% of individuals so longitudinal studies of chemical AD biomarkers can be conducted over a 3-year observation period. Data from periodic clinical evaluations will facilitate the correlation of neuroimaging and biomarker findings with neuropsychological and behavioural data. To accelerate achieving these goals, all data collected from ADNI subjects is made publicly accessible. Significantly, ADNI recruitment reached the halfway point in August 2006 and recruitment will be complete in 2007.

To implement the ADNI mission, the ADNI Biomarker Core at the University of Pennsylvania, USA, collects and banks all biological samples (DNA, blood, urine and CSF) from all participating sites, and conducts studies of selected AD biomarkers, including *APOE* genotype, isoprostanes, tau, Aβ, sulphatides and homocysteine. Although these analytes were selected for study in the Penn Biomarker Core based on a consensus of AD biomarker experts[24], this Core will make banked ADNI biosamples available for studies of additional biomarkers by other investigators according to procedures outlined on the ADNI website. A brief overview and update of the status of tau, Aβ and isoprostanes as AD biomarkers is provided in the main text.

subjects[35]. So, predicting which patients are at greater risk for conversion to AD is aided by *APOE* genotyping, but this information contributes little towards establishing a 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 Aβ and tau, plasma homocysteine measurements and *APOE* genotyping have the potential to further improve the diagnosis of AD as part of a panel of AD biomarkers.

Future directions

There is increasing evidence that a number of potentially informative AD biomarkers can improve the accuracy of diagnosing AD, especially when they are used as a panel of diagnostic assays and interpreted in the context of neuroimaging and clinical data[23–31]. However, further studies are needed that use fully bioanalytically validated immunoassays and other test formats, as proposed in the ADNI[31], but studies are also needed that follow patients longitudinally to autopsy in order to correlate biomarker findings with definitive neuropathological diagnoses[36].

Nonetheless, there is reason to be optimistic that a validated panel of AD biomarkers will be the outcome of the ADNI and other research programmes[25–29,31]. The successful accomplishment of this goal will facilitate the reliable diagnosis of AD in its early stages or even in its prodromal stages, as well as provide tests for reliably distinguishing AD from other forms of dementia. Indeed, it would also be attractive if diagnostic AD biomarker tests could be effectively introduced for general medical practice in various routine clinical settings, and a recent study suggests this could be the case[37]. Making such tests widely available to non-specialists will provide them with objective data from AD biomarker assays to assist in making more informed decisions about referring patients with questionable cognitive impairments to specialty clinics for further evaluation in a timely manner. This will be especially important with the development of more effective AD-specific therapies, the availability of which will increase the urgency of finding tests that distinguish AD from other forms of dementia that require different therapeutic interventions. For example, the application of an AD CSF biomarker panel of tau, Aβ1–42 and 8,12-iso-iPF$_{2\alpha}$-VI to a cohort of FTD patients and controls suggested that this panel can distinguish FTD from AD because the values for these CSF biomarkers in FTD patients were more similar to controls than to AD patients[38]. However, it would also be useful to have informative FTD-specific biomarkers, as they could enhance the accuracy of diagnosing FTD and distinguishing it from AD. Although considerable research is needed to accomplish this goal, FTD-specific biomarkers could emerge from further advances in understanding mechanisms of FTD brain degeneration, including the role of ubiquitinated TAR DNA-binding protein 43 (TDP-43) inclusions[39] and progranulin gene (*PGRN*) mutations[40,41] in the pathogenesis of FTD. In addition, dementia with LB-specific and LB variant of AD-specific biomarkers would be similarly informative. These could be grounded in a better understanding of the pathobiology of α-synuclein inclusions in these disorders and they could result from the development of sensitive ELISAs to measure α-synuclein in CSF or blood[42]. Moreover, by combining the power of CSF biomarkers such as tau and Aβ with neuroimaging techniques to visualize Aβ deposits (or accumulations of other neurodegenerative disease lesions), it might be possible to identify individuals at greatest risk for developing MCI and converting to AD[43].

Nonetheless, although hypothesis-driven candidate biomarkers such as those mentioned above should continue to be the focus of AD biomarker research, it is timely to pursue the identification of biomarkers using unbiased strategies based on proteomics, metabolomics or related technologies[44–46]. Furthermore, a better understanding of the metabolism and turnover of AD biomarkers, as well as diurnal variations in their levels in various bodily fluids, will greatly facilitate the interpretation of assays of individual analytes, as well as combinations of analytes, for use in diagnostic and other applications[47]. So, the unique public–private commitment to implement ADNI underlines the importance of the public health benefits that will come from validating informative biomarkers to translate laboratory advances in understanding mechanisms of AD brain degeneration into better diagnostic strategies and accelerate the pace of developing more meaningful disease-modifying therapies for AD and related neurodegenerative disorders.

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Competing interests statement
The author declares no competing financial interests.

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