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Review

Biomarkers for Preclinical Alzheimer's Disease

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Abstract. Currently, there is a pressing need to shift the focus to accurate detection of the earliest phase of increasingly preclinical Alzheimer's disease (AD). Meanwhile, the growing recognition that the pathophysiological process of AD begins many years prior to clinically obvious symptoms and the concept of a presymptomatic or preclinical stage of AD are becoming more widely accepted. Advances in clinical identification of new measurements will be critical not only in the discovery of sensitive, specific, and reliable biomarkers of preclinical AD but also in the development of tests that will aid in the early detection and differential diagnosis of dementia and in monitoring disease progression. The goal of this review is to provide an overview of biomarkers for preclinical AD, with emphasis on neuroimaging and neurochemical biomarkers. We conclude with a discussion of emergent directions for AD biomarker research.

Keywords: Biomarker, blood, cerebrospinal fluid biomarkers, clinical biomarkers, genetic biomarkers, neuroimaging, preclinical Alzheimer's disease

INTRODUCTION

Alzheimer's disease (AD), the most common form of dementia, has an insidious onset with progressive deterioration in cognition, functional ability, behavior, and mood. This age-associated neurodegenerative disorder influences more than 36 million people worldwide. Moreover, it has been predicted that by 2050, this figure will more than 115 million [1]. Although current available interventions can provide benefits by the time the diagnosis of AD is made, none prevents or cures the disease [2]. However, the pathological process leading to AD begins decades before the typical motor symptoms, and there is a growing conviction that interventions must commence at the earliest possible stage of the disease to have as positive an impact on the illness as possible. Thus, there is a pressing need to shift the diagnostic focus toward the detection of increasingly preclinical AD not only to advance our understanding of the disorder but also to provide better tools for early diagnosis when neuroprotection is possible.

Preclinical AD can be defined as a long continuum that AD neuropathological abnormalities begin to accumulate but cognitive ability is normal [3–6]. As tools that assist in detection and diagnosis of the disease, biomarkers for preclinical AD have become an area of great interest for both clinicians and researchers. Now that biomarkers for AD have become

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available, identification of preclinical AD *in vivo* in cognitively normal individuals is possible. In this review, we will focus on the current research on the biomarkers for preclinical AD—the use of clinical assessment, neurochemical biomarkers, neuroimaging, genetic, and other biomarkers. We also share our opinions regarding the requirements for further progress in this area.

PRECLINICAL STAGES OF AD

To advance the study of preclinical AD, the Preclinical Working Group of the National Institute on Aging (NIA) and Alzheimer's Association (AA) proposed a conceptual framework and operational research criteria for preclinical AD [7]. This new and earlier predementia condition represents a successive course from completely asymptomatic individuals with biomarker evidence of AD pathophysiologic changes to biomarker-positive individuals who are already demonstrating very subtle decline but not yet meeting standardized criteria for mild cognitive impairment (MCI) (Fig. 1). MCI, a clinical construct immediately preceding AD dementia, corresponds to an obvious prodromal stage of the disease.

The NIA-AA criteria for preclinical AD are conceptualized as having 3 stages (Fig. 2). The individuals in stage 1 have biomarker evidence of Aβ which can be demonstrated by positron emission tomography (PET) amyloid imaging or cerebrospinal fluid (CSF) amyloid-β (Aβ) levels, but no detectable evidence of additional brain alterations suggestive of neurodegeneration or subtle cognitive and/or behavioral symptomatology. Stage 2 is characterized by amyloid positivity and, in addition, neuronal injury markers as evidenced by brain atrophy on structural MRI, hypometabolism on ^{18}F-fluorodeoxyglucose (FDG) PET, or elevated levels of CSF tau. In the last stage of preclinical AD (stage 3), the individuals have biomarker evidence of amyloid accumulation, early

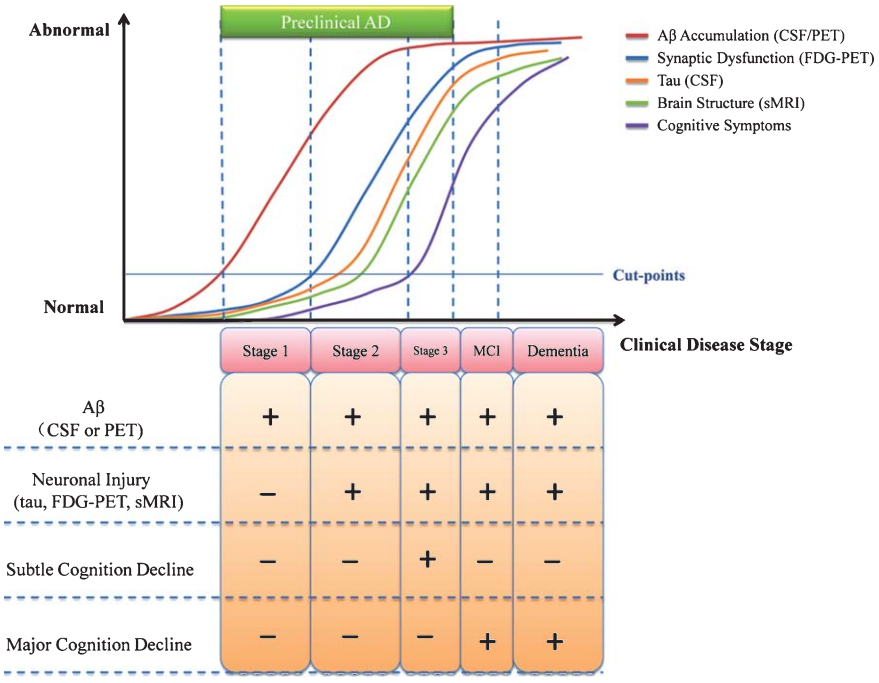

Fig. 1. Hypothetical model of dynamic biomarkers of the preclinical AD. The horizontal axis indicates clinical stages of AD: preclinical AD, mild cognitive impairment (MCI), and dementia. The vertical axis indicates the changing values of each biomarker, scaled from maximally normal (bottom) to maximally abnormal (top). Aβ is identified by cerebrospinal fluid (CSF) $A\beta_{42}$ assay or PET amyloid imaging. Synaptic dysfunction evidenced by fluorodeoxyglucose (F18) positron emission tomography (FDG-PET) or functional magnetic resonance imaging (fMRI). Neuronal injury is evidenced by CSF tau or p-tau, brain structure is evidenced by structural magnetic resonance imaging (sMRI). The horizontal "cut-points" line represents the cut-points used to operationalize preclinical staging. This figure represents a theoretical framework for the ordering and dynamic sensitivity of various AD biomarkers across the pathologic and clinical AD spectrum. Aβ-plaque biomarkers are dynamic early in the disease, before the appearance of clinical symptoms, and have largely reached a plateau by the time clinical symptoms appear. Biomarkers of synaptic dysfunction are dynamic later in the disease and correlate with clinical symptom severity. MRI and cognitive symptom are the last biomarker to become abnormal. Rates of change in each biomarker change over time and follow a non-linear time course, which we hypothesize to be sigmoid shaped. This figure is actually an upgraded version of Fig. 1 in Jack et al. [8].

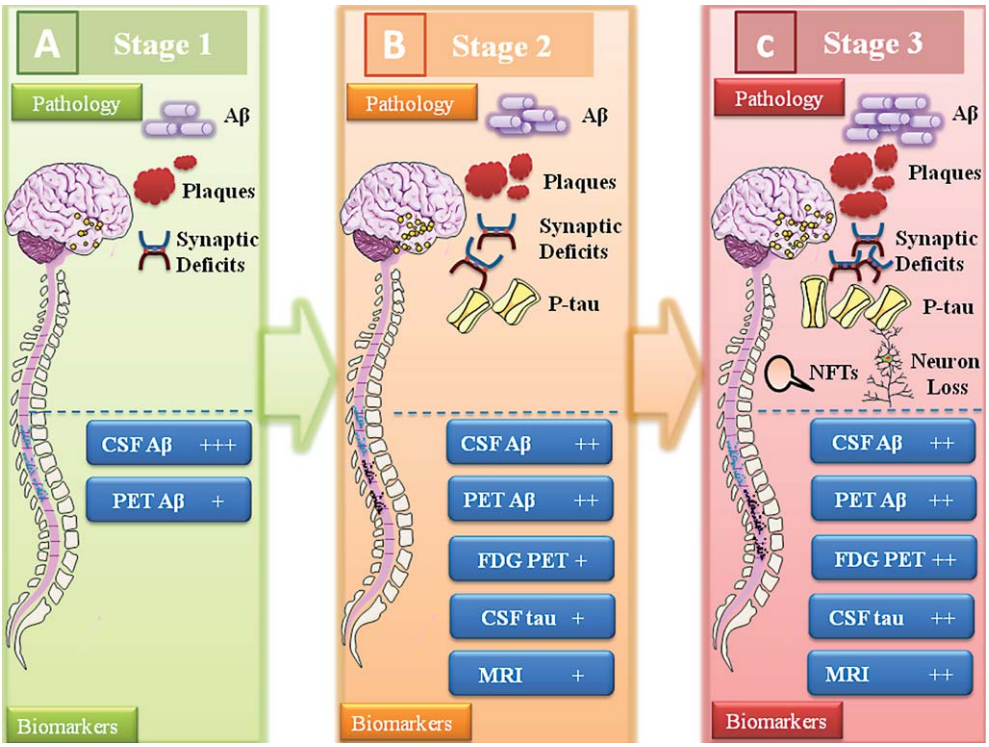

Fig. 2. The brain pathology and biomarkers of preclinical AD. The preclinical stage of AD can be further subdivided into three stages: stage 1 (the stage of asymptomatic cerebral amyloidosis), stage 2 (amyloid deposition plus evidence of synaptic dysfunction and/or early neurodegeneration), and stage 3 (amyloid deposition plus evidence of neurodegeneration).

neurodegeneration, as well as the evidence of subtle cognitive decline [7]. The order of the NIA-AA stages promotes increasing studies to investigate the prevalence and long-term outcome of preclinical AD according to these criteria, and ultimately, aids the field in moving toward earlier intervention.

Although the NIA-AA criteria are based on observational data, they make specific assumptions about relationships among biomarkers and cognitive testing that have not been adequately validated. Hitherto emerging data imply a number of issues that deserve further consideration. It is evident that two additional categories are necessary to classify all cognitively normal subjects: stage 0 to denote those cognitively normal subjects with no biomarker evidence of AD pathophysiology and no evidence of subtle cognitive impairment, and a category termed Suspected Non-Alzheimer Pathology (SNAP) consists of subjects with normal amyloid PET but one or both neuronal injury biomarkers abnormal. The SNAP group could represent subjects in this category harbor the apolipoprotein E (APOE) $\varepsilon 4$ gene—a key risk factor for AD. Additionally, there are a small number of subjects are labeled "unclassified", because they fail to fit into any group [8].

CLINICAL BIOMARKERS FOR PRECLINICAL AD

Substantial evidence suggests that a variety of symptoms can precede the classic features of AD. Symptoms, suggested as possible premonitory features of AD, include cognitive impairment, loss of function and worse sleep quality.

Cognitive impairment

As subtle cognitive change turns up in the later stage of subtle cognitive decline, it is possible open a small diagnostic window between the curve of cognitive decline in normal aging and the entrance to the preclinical stage. Increasingly, it has been suggested that subtle cognition decline, at the stage of preclinical AD, may indicate initial cognitive decrements in cognitively normal individuals that are otherwise undetectable with standardized objective tests of cog-

nitive performance [9]. Moreover, evidence points to the fact that preclinical AD is characterized by numerous impairments affecting multiple cognitive domains, including episodic memory, verbal abilities and learning, visuospatial function, attention, and executive functions [10].

More sensitive cognitive measures, particularly with challenging episodic memory measures (such as delayed recall of word lists and paired-associates learning), may detect very subtle cognitive impairment in amyloid-positive individuals to predict the subsequent AD. An initial analyses of a familial AD cohort [the Dominantly Inherited Alzheimer Network (DIAN)] suggests that declines in performance on tests of episodic memory accelerated approximately 10 years so before expected symptoms of AD, implying that measured change in cognition overtime will be more sensitive than any one-time measure [11]. Moreover, recent study confirms that high levels of Aβ were associated with greater decline in episodic memory measures over 6 months in healthy older adults [12]. Simultaneously, a deficit in face-name paired associate learning was recently observed in non-demented elderly individuals with Aβ burden in brain regions associated with memory systems, suggesting that a deficit in associative memory may also be a a sensitive marker of early marker of AD [13]. In addition, familiarity-based memory has generally been considered to be spared during normal aging, but it remains controversial whether this type of memory is impaired in early AD. Recent study provides promising data to support that familiarity may be a sensitive biomarker of AD-specific brain changes in preclinical AD and that it may offer a qualitatively distinct measure of early AD memory impairment relative to normal age-associated change [14].

CSF evidence of preclinical AD in patients with subjective complaints predicted cognitive decline over time, including more than memory alone. Executive functions also deteriorated [15]. Measures of executive function included Category Fluency (animals, fruits, vegetables), Letter Fluency (FAS), and tests that are sensitive to prevalent and incident dementia. Moreover, asymptomatic longitudinal executive function decline is associated with subsequent increased fibrillar amyloid deposition, even when controlling for APOE ε4 genotype [16]. A recent prospective longitudinal study which used five measures of executive function suggests that measures assessing inhibition and switching abilities provide predictive utility of subsequent global cognitive decline independent of episodic memory [17]. Moreover, another unexpected result that decreased executive performance before memory impairment in preclinical AD urges longitudinal designs to more fully evaluate this approach [18]. Such cognitive measures, if reliable, are potentially of considerable value as a sample but effective screening tool to further determine who should go on to conduct more specific molecular or structural tests at a very early stage.

However, there have been few studies of the overall latent patterns of cognitive performance in normal individuals across different cognitive domains currently. Recent study using data from the National Alzheimer Disease Coordinating Center demonstrate the use of latent profile analysis for pre-clinical phenotypes of AD defined by cross-sectional patterns of cognitive performance rather than cognitive decline or performance on a single test of global cognitive function [19], elucidating it is a useful method to evaluate patterns of cognition for the identification of preclinical AD phenotypes.

Worse sleep quality

As sleep and circadian problems are very common in AD, we speculate whether sleep abnormalities are present in the preclinical AD, prior to the manifestation of any cognitive impairment. In a mouse model of Aβ accumulation, sleep-wake cycles became highly fragmented following formation of amyloid plaques [20]. Moreover, the sleep-wake cycle, which appear to be a fundamental property of the brain, are likely disrupted by Aβ aggregation in human brain other than the effect of aging [20]. Several cross-sectional studies concluded that sleep disturbance was associated with poor cognitive function [21], reminding us that sleep quality in older adults should receive particular attention by clinicians as poor sleep quality can be an early sign of cognitive decline. Another recent cross-sectional study supported that amyloid deposition in the preclinical stage of AD appears to be associated with worse sleep quality, thereby implying that sleep measures could be used as biomarkers of brain function to facilitate faster and easier clinical trials of promising treatments in the preclinical AD [22]. Moreover, sleep disturbances was associated with an increased risk of developing AD, and remained a strong factor for dementia when overall health status was added to the risk model [23]. In summary, sleep disturbance may exist prior to the manifestation of other typical symptoms observed in AD (e.g., memory loss), thereby it may be useful for screening individuals at risk for AD and may allow for the earlier detection of AD at the preclinical stage.

NEUROCHEMICAL BIOMARKERS FOR PRECLINICAL AD

Cerebrospinal fluid biomarkers

Even though a definite diagnosis of AD can be formulated only neuropathologically, CSF markers play a supportive role in the diagnosis of probable AD. Moreover, abnormal levels of biomarkers in CSF are assumed to be active long before the first symptoms appear. Thus, considerable research effort has been invested in evaluating the claim that these biomarkers can detect preclinical AD before behavioral symptoms arise [24].

Aβ is produced in the brain and diffuses into the CSF, appearing in moderate concentrations with multiple forms in cognitively normal individuals. Among the kinds of species, $A\beta_{42}$ seems to be essential for initiating Aβ aggregation and is considered as a useful biomarker for AD. However, the mean concentration of $A\beta_{42}$ in the CSF is significantly reduced in subjects with AD relative to age-matched controls, which is inversely correlated with Aβ burden [25]. Relevant longitudinal studies of CSF also demonstrated that $A\beta_{42}$ can serve as a diagnostic and surrogate biomarker for $A\beta_{42}$ deposition in the brain [26]. Recently, a powerful meta-analysis supported that increased Aβ burden has small but nontrivial associations with specific domains of cognitive performance in individuals who are currently cognitively normal, implying that $A\beta_{42}$ can be useful for identifying preclinical AD or developing clinical outcome measures [27]. Moreover, low levels of $A\beta_{42}$ appear early in the course of AD, and even earlier, in people who are still cognitively normal which precedes MCI by several years [28–30]. Recent data are consistent with a decline in CSF $A\beta_{42}$ levels occurring at least 20 years prior to clinical dementia in individuals with familial AD mutations [31]. However, it is a pity that $A\beta_{42}$ can be seen in other dementias, potentially limiting the utility of $A\beta_{42}$ alone in differentiating AD from other dementias. Thus it should be combined with other biomarkers to predict the specific dementia.

By contrast to $A\beta_{42}$, the CSF tau levels reflect the progression of tau-related pathology within the cerebral cortex. Tau is elevated in the CSF in most patients with AD and reflects the neuronal loss associated with the physiopathological process of AD [32]. Similarly, tau elevation seems to occur at some cognitively normal individuals, where its levels correlate with the amount of amyloid deposition and together with $A\beta_{42}$ predict cognitive decline [24, 33]. Just as $A\beta_{42}$, it is important to consider that total tau (t-tau) elevation alone is less useful in the differential diagnosis of AD. However, a bioassay of phosphorylated (p)-tau demonstrated a sensitivity of 85% and a specificity of 97% in distinguishing AD from other neurological disorders. These preliminary data suggest that p-tau is superior to t-tau in differentiating diagnosis, thus making up the shortage of $A\beta_{42}$ and t-tau in differentiating diagnosis [34–36]. Moreover, the decrease of p-tau-181 appears to correlate with cognitive functioning [37]. Significant disruptions in CSF t-tau and p-tau metabolism likely occur after $A\beta_{42}$ initially aggregates and increases as amyloid accumulates [38]. However, there is still limited evidence of this relationship in human studies. Further longitudinal biomarker studies of non-demented individuals with extended clinical follow-up are needed to provide more details about this relationship.

The default mode network (DMN), which is a collection of interconnected brain regions that maintain robust metabolic activity when the brain is in a resting state, are among the regions earliest affected in AD [39]. Recently, studies have confirmed that both decreased CSF $A\beta_{42}$ and increased CSF phosphorylated tau independently affect DMN integrity among older adults with normal cognition [40]. Based on these studies, it is feasible to combine CSF $A\beta_{42}$ with t-tau or p-tau to predict progression from cognitive normalcy to MCI/very mild dementia. Several powerful studies support that CSF tau/$A\beta_{42}$ and p-tau/$A\beta_{42}$ ratio in normal individuals has been associated with an increased risk of cognitive decline, implying that the CSF tau/$A\beta_{42}$ ratio is a very good marker of predicting the dementia over a 3 to 4 years period [41]. Moreover, compared to the biomarkers themselves, the $A\beta_{42}$/tau ratio can further increases its sensitivity and specificity in detecting symptomatic AD and differentiating it from frontotemporal dementia [42, 43]. Therefore, it appears that the normal elderly individuals with a high ratio of tau/$A\beta_{42}$ represent individuals with earliest preclinical AD.

In addition, converging evidence points that AD is associated with alterations in bioenergetics and mitochondrial function [44, 45]. Neurons are highly dependent on aerobic energy provided by mitochondria, which contain several copies of their own DNA (mtDNA). The particular covalently closed circular form of mtDNA renders it more resistant to degradation by nucleases, thereby possibly presenting in the CSF. This noteworthy feature reminds us to explore whether it is possible to detect cell-free mtDNA in the CSF, ultimately reflect alterations in brain metabolism and

might represent an early indicator of the neurodegenerative process in AD. Recent studies exactly support this hypothesis that low content of mtDNA in CSF may be a novel biomarker for the early detection of preclinical AD [46]. More longitudinal studies of CSF biomarker dynamics are needed, especially in patients during the preclinical stage of the disease.

In addition, the occurrence of a plaque-dependent inflammation in AD has been extensively documented in both human specimens and transgenic models of the disease. And several inflammatory markers have also been proposed as AD biomarkers in previous literature [47]. Mediators that are upregulated during the earliest, oligomeric-induced inflammatory process in AD brains would represent ideal markers for early diagnosis. A promising candidate in this regard is matrix metalloproteinase 9 (MMP-9). MMP-9, a member of the family of Zn^{2+}-containing and Ca^{2+}-requiring endoproteases, plays a role in normal tissue remodeling event. Pathological upregulation of MMP-9 levels is associated, during inflammatory processes, with infiltration of white blood cells and blood-brain barrier damage [48]. Advanced studies demonstrated that upregulation of cerebral levels of MMP-9 activity is an early event, as MMP-9 levels are already significantly increased in CSF of elderly individuals might represent a novel biomarker of early detection of AD [49].

Blood-based biomarkers

Plasma and serum biomarkers reflect the underlying pathophysiological process of senile plaque formation, including Aβ protein, Aβ autoantibodies, platelet amyloid-β protein precursor (AβPP) isoforms. Compared with the high cost and limited availability of PET imaging and the invasive and specialist nature of CSF collection, these blood samples are more accessible to the wider communities.

Research into the AD-affected blood proteome has focused on Aβ measurements in cellular fractions, serum, and plasma [50]. However, studies on Aβ in plasma are contradictory, and show very marginal differences between patients and controls. Although there is suggestive evidence that changes in plasma $A\beta_{40}$, $A\beta_{42}$ may be associated with individuals at risk for developing AD [51], current cross-sectional analysis and longitudinal cohort studies provides no support for the usefulness of plasma Aβ as a diagnostic marker for early AD, suggesting the insufficient evidence of plasma $A\beta_{40}$, $A\beta_{42}$, or the ratio of $A\beta_{42}/A\beta_{40}$ serving as biomarkers for preclinical AD [52, 53]. Cohort studies have yielded equally conflicting results, with higher baseline levels of $A\beta_{42}$ [54, 55] in those with worsening cognition, whereas other studies have reported lower plasma $A\beta_{42}$ levels [56]. Results from the Alzheimer's Disease Neuroimaging Initiative (ADNI) indicated that plasma levels of both $A\beta_{40}$ and $A\beta_{42}$ showed modest value as prognostic markers [55]. Recently, studies suggest that although the prognostic value of individual measures in any given subject is limited, the diagnostic contribution of plasma Aβ may demonstrate utility when combined with a panel of peripheral biomarkers [57]. This finding implies that further enhancement of plasma Aβ assays may be effective in the identification of at-risk individuals in large-scale screening. Univariate analysis of ADNI studies show that a panel of blood-based biomarkers is able to accurately predict neocortical Aβ (extracellular Aβ) burden, supporting the hypothesis for a relationship between a blood-based signature and Aβ accumulation [58].

In addition, recent evidences that human serum contains stably expressed microRNAs (miRNAs) have revealed a great potential of serum miRNA profile that can be used as a fingerprint for the diagnosis of AD [59, 60]. Increasing studies identified six serum miRNAs that distinguish AD patients from healthy controls with high sensitivity and specificity, suggesting that serum miRNA panel (or miR-342-3p alone) may serve as a novel biomarker for preclinical AD [61]. Thus, although there is no feasible blood biomarker in predicting AD conversion especially in relation to sensitivity and specificity based on current insufficient data, it is conceivable that combinations of plasma biomarkers can be used as surrogate indicators of disease risk for AD [62].

NEUROIMAGING BIOMARKERS FOR PRECLINICAL AD

Neuroimaging techniques have increasingly been used to detect brain changes associated with AD, and thus have potential as noninvasive biomarkers for preclinical AD [63].

Magnetic resonance imaging (MRI)

Structural MRI

Abnormalities in structural MRI become detectable earlier in the asymptomatic phase, and thus might serve as a predictor of future dementia prior to symptoms. Structural MRI studies in subjects diagnosed with AD or MCI consistently show atrophy in the entorhinal cortex and hippocampus, thus gaining a broad consensus

that the atrophy of the medial temporal lobe (MTL) and cortical thinning in certain AD-vulnerable regions are the first MRI signs of emerging AD [64, 65]. Moreover, the evidence of tight association between severity of MTL atrophy assessed with MRI scans and severity of MTL degenerative pathology at autopsy suggest that MTL atrophy can be used as a criterion in the diagnosis of probable AD [66]. MRI studies focused their attention on normal subjects at genetic risk of developing AD demonstrated that cognitively normal subjects with maternal history of AD have reduced volume in several brain regions, including MTL and precuneus [67, 68]. Recent studies using a whole-brain voxel-based approach determined that structural MRI measurement methods could be used to identify the presence of brain atrophy in specific cortical regions up to 10 years before clinical symptoms of AD occur, with a greater relevance in the MTL [69]. Further studies suggest that quantitative MRI and proton magnetic resonance spectroscopy (MRS) markers predict progression to AD and cognitive decline in cognitively normal older adults [70]. Moreover, proton MRS may contribute to the assessment of preclinical AD pathologies by capturing neurodegenerative changes that are not detected by hippocampal volumetry. Although the exact time of cortical atrophy varies greatly over studies, there is broad consensus that the subtle but reliable atrophy in hippocampus can be a potentially important imaging biomarker for preclinical AD. Simultaneously, this measure can improve the sensitivity and specificity of detecting AD at early stages, especially when combined with cognitive measures [71].

In addition, even at this pre-dementia stage of the disorder, atrophy is not restricted to medial temporal areas, but extends to widespread areas of association cortex. Other measures such as global measures of atrophy, such as reduced global brain volumes and increased ventricular volumes have also been described in cognitively normal individuals at risk for AD [72]. In individuals which represent an earlier stage of the disorder, significant atrophy appears to the areas beyond the medial temporal lobe, including thinning of lateral temporal, posterior cingulate, inferior parietal, precuneus, and caudal middle frontal cortex [73]. In univariable modeling, white matter hyperintensity volume in the parietal lobe was significant predictors of AD in cognitively normal older adults [70, 74]. In addition, MRI cortical thickness represents as a biomarker that has a relatively high likelihood of imminent cognitive decline consistent with prodromal AD in normal adults [75]. Thus, it is possible to improve the accuracy of structural MRI though including the measures of atrophy beyond the medial temporal lobe into the analysis.

Functional MRI

Resting state functional connectivity functional MRI (rs-fcMRI) analysis is a relatively new province of biomarkers for AD and is increasingly used to detect subtle brain network abnormalities in preclinical AD. Several studies using fMRI have supplied supporting evidence demonstrating that, as in AD and MCI, there is significantly decreased DMN connectivity in cognitively normal elderly persons with elevated brain amyloid [76–78]. In these cognitively normal individuals, resting state functional connectivity of the precuneus (part of DMN) was significantly decreased with the left hippocampus, anterior cingulate cortex, and gyrus rectus, and increased with visual cortex. Another study built on the notion of brain "hubs" (brain regions typically highly connected to multiple other brain areas) to investigate the effects of amyloid, demonstrating that older individuals with increased brain amyloid burden had disruptions of functional connectivity in cortical hubs (e.g., precuneus) in non-demented subjects and that connectivity disruptions were associated with hypometabolism [79]. The spatial overlap between hypometabolism and disruption of connectivity in cortical hubs pointed to particular susceptibility of these regions to early changes on the path to AD. Another recent study demonstrated that graph theoretical measures applied to rs-fcMRI data can define changes because of AD, and that changes are present in cognitively normal participants with CSF measures indicative of preclinical AD [80]. Moreover, the robust changes seen in functional imaging studies of normal aging, MCI, and AD provide the field with a potentially sensitive, non-invasive biomarker that is closely linked to the neural machinery of memory. Going forward, the hope is that these promising fMRI techniques can be used to complement more traditional CSF and serum biomarkers to facilitate earlier diagnosis of AD [81, 82]. At the same time, fMRI show that the magnitude of brain activation in the parietal and prefrontal regions of cognitively normal APOEε4 carriers during memory tasks is higher than in controls, and the extent of brain activation correlates with subsequent memory decline in these subjects, reflecting a compensatory response to accumulating AD pathology [83]. Although these findings are inconsistent in AD, the compensatory increase in extrahippocampal activity in fMRI should be considered as an early functional sign of emerging AD in cognitively normal individuals [84]. Recent studies demonstrate that subtle changes

in functional activity of the precuneus and posterior cingulate cortex during encoding measured by fMRI precede clinical and cognitive symptoms in the AD continuum, providing evidence insinuating that it may be a suitable biomarker of preclinical AD [85].

Positron emission tomography

PET with Pittsburgh compound B

With the development of PET imaging of the amyloid-binding agent Pittsburgh compound B (PET-PiB), it is possible to quantify the brain Aβ load and its spatial distribution that were previously restricted to postmortem studies.

The pooled analysis of five longitudinal studies provided sufficient evidence for a direct relationship between PET-PiB and the likelihood of conversion from a clinical diagnosis of MCI to a clinical diagnosis of AD dementia over 3 years [86]. Similar recent prospective data show that Aβ positivity, as detected by PET-PiB neuroimaging, is associated with substantial decline in episodic memory decline over 18 and 36 months in healthy older adults, even in the absence of any change in clinical disease status [12, 87, 88]. Compared with PiB-negative individuals, these PiB-positive cognitively normal subjects have slightly lower cognitive performance including very subtle episodic memory impairment, smaller hippocampus volume, and accelerated rate of cortical atrophy [89, 90]. Longitudinal studies assessing the relationships between baseline Aβ deposition and subsequent changes in cognition or brain volume showed that 5 out of 32 (16%) of the PiB-positive cognitively normal elderly developed MCI by 20 months while only one out of 73 PiB-negative normal elderly developed MCI, suggesting that the presence of Aβ deposition in the brain of cognitively normal elderly is associated with a worse prognosis [87, 91]. As the current data are still not overwhelmingly significant, we can only draw a conservative conclusion though these limited evidences of an association between PET-PiB positivity and the likelihood of conversion from normal cognition to a clinical diagnosis of MCI [86].

On the other hand, these PiB-positive people clearly represent a subpopulation at risk for dementia. Among cognitively normal elderly, 49% of APOE ε4 carriers were PiB-positive while they were only 21% within the non-carriers [92]. The higher prevalence of PiB positivity among cognitively normal individuals with known genetic AD risk factors further support the idea that increased PiB retention may serve as a predictive factor of AD-type dementia in healthy older individuals [93].

As there is significant overlap between the utility of amyloid imaging and measurement of CSF $A\beta_{42}$ as a screening tool, researchers attempt to address the areas where these two biomarkers may be equivalent and areas where one measurement may hold unique advantages [94]. However, whether PiB uptake might be more sensitive than CSF $A\beta_{42}$ concentration in detecting increased amyloid burden remains unclear. Thus, more follow-up studies are needed to confirm this confused issue.

In addition, recent studies also support the hypothesis that higher amyloid burden assessed by florbetapir 18F (^{18}F-AV-45) amyloid PET is associated with lower memory performance among clinically normal older subjects [95]. Longitudinal follow-up of the present and other cohorts imaged with florbetapir F18 are ongoing to determine whether ^{18}F-AV-45 may also predict subsequent cognitive decline.

Fluro-D-glucose PET

Assessment of regional cerebral glucose metabolism by PET imaging with 2-deoxy-2[18F]fluoro-D-glucose as a tracer (FDG)-PET at resting state is a standard functional technique to assess cerebral function. Accumulating scientific evidence demonstrated that regional functional impairment of resting glucose metabolism in AD is closely related to the severity, progression and type of cognitive deficits [96]. Moreover, FDG-PET can differentiate between AD and frontotemporal dementia with high accuracy (90%) [97]. Thus, it is of interest to determine whether FDG-PET could improve diagnostic accuracy in preclinical or prodromal AD. Previous studies showed that medial temporal lobe FDG-PET hypometabolism was associated with subsequent conversion to MCI or AD dementia [98, 99]. Numerous studies has shown that temporoparietal association areas in a group of asymptomatic individuals at high risk for AD due to family history of AD and the APOE ε4 allele expressed reduced metabolic rates, and this abnormality was seen decades before the probable onset of dementia [100]. Cross-sectional analyses of a familial AD cohort (DIAN) suggest that a significant decrease in cerebral metabolism in the precuneus was detected in mutation carriers 10 years before expected symptom onset [11]. Recent studies which focus on the prediction of the conversion from cognitive healthy subjects to MCI and AD dementia based on the *a priori* determined FDG-PET (located in the medial temporal and parietal lobes) is consistent with previous studies showing that reduced medial temporal and parietal FDG-PET was associated with faster cognitive decline and hippocam-

pal/entorhinal FDG-PET was sensitive to preclinical AD [101].

In addition, although FDG-PET can provide closer relation to clinical symptoms, it is less sensitive than amyloid imaging remained below a clinically significant level for the prediction of progression to AD, especially in preclinical disease. Moreover, the exact pathophysiological significance of the reduced cerebral glucose metabolism in cognitively normal individuals is difficult to interpret. Thus, in-depth analysis of longitudinal studies and increasing use of FDG-PET in clinical trials as an additional outcome parameter will increase knowledge and confidence about its properties as a biomarker for preclinical AD.

GENETIC BIOMARKERS FOR PRECLINICAL AD

Although the presymptomatic phase of AD has not been well characterized, there is a general consensus that neuronal damage, as a result of complex genetic, environmental, and other factors, proceeds years or even decades before the onset of symptoms. Understanding these interactions may identify early biomarkers of degeneration and aid in preclinical screening. Lahiri and his colleagues proposed a 'Latent Early-life Associated Regulation' (LEARn) model, positing latent changes in expression of specific genes initially primed at the developmental stage of life [102, 103]. Under LEARn, early–life influences, such as exposure to metals, nutritional variation, variation in maternal care, and other stressors modify potential expression levels of disorder–associated genes in a latent way. Moreover, the majority of AD cases would follow an etiology based on this model [104]. Studies on primates and rodents has determined that early life exposure to environmental metal, such as lead, may induce a short-term upregulation of AβPP and Aβ in the aging brain, followed by a long latency period of 'normal' levels of gene expression, but this period ends when disease-associated gene expression levels increase later in life [105, 106]. In addition, nutrition plays a vital role in methylation of DNA, which is well associated with pathophysiological processes of AD [107, 108]. Further B-vitamin supplementation trials focusing on elderly subjects with high homocysteine levels are warranted to see if progression to dementia can be prevented [109]. These facts suggest that environmental factors, including exposure to risk agents such as heavy metals, nutritional imbalance, and stress contribute to presentation of AD in a long latent period. Latent changes in these genes are maintained by epigenetic mechanisms such as DNA methylation, DNA oxidation, and chromatin reorganization. This model may provide the use of early dietary and other lifestyle supplementation as another prophylactic measure against AD, directing research toward discovering early–life epigenetic changes that associate with AD to eliminate AD before it occurs. Ultimately, more effective later–life prophylactic and remediation that would reduce the need for "catch–up" symptom–based therapies may contribute to the public health practices.

APOE polymorphism, as a major risk factor for AD, is involved in lipid and cholesterol transport, cell repair, and Aβ deposition and certain studies suggest potential implications in neurogenesis [110]. The study conducted by the Alzheimer's Disease Cooperative Study examined the impact of age and APOE genotype on the rate of cognitive decline in non-demented elderly participants, suggesting that preclinical disease may be overrepresented in older APOE $\varepsilon 4+$ individuals [111]. Moreover, recent cross-sectional comparisons supported that preclinical AD is characterized by a significant APOE $\varepsilon 4$ gene dose effect that was confined to the memory domain and the Dementia Rating Scale [112]. However, although possession of the APOE $\varepsilon 4$ allele is the strongest risk factor for AD in late middle age, it does not have preclinical effects early in the lifespan on cognitive functions [113]. Moreover, the absence of its prognostic utility in individuals with AD pathological abnormalities is consistent with findings from previous studies [114]. This peculiar situation reminds us that the distinctions between genetic risk factors for AD versus biomarkers of AD should be recognized in the context of risk prediction. Among them, the greatest difference is that genetic status remains constant throughout life, while biomarkers are dynamic as they reflect the current state of pathophysiology [115]. Thus, APOE is considered to be a major risk factor for AD other than a standard biomarker for preclinical AD [116].

In addition to the APOE gene, previous genome-wide association studies in individuals of European ancestry identified nine other genomic regions associated with late-onset AD (LOAD), including BIN1, CLU, CR1, PICALM, ABCA7, MS4A6A, EPHA1, CD33, CD2AP, PLD3 [117–125]. The four susceptibility loci BIN1, CLU, CR1, PICALM is verified to be the most promising genomic regions associated with LOAD, while CD33, MS4A6A/MS4A4E, EPHA1, CD2AP, ABCA7 also confirmed their relationship with AD in subsequent repeated trials [126, 127]. Recent studies demonstrate that several genotype patterns, including CR1, BIN1, CLU, PICALM,

influence episodic memory performance, and the pattern that included PICALM and CLU was the strongest genotypic profile for lower memory performance, a surrogate indicator of LOAD [128, 129]. Thus, Identifying genotypic patterns contributing to the decline of an individual's cognitive performance may be a critical step along the road to preclinical detection of AD. Recently, heterozygous rare variants in TREM2 were identified to be associated with a significant increase in the risk of AD except in the northern Han Chinese population [130–133]. Detection of mutations in such prospective genes, identified as the major cause of LOAD, may serve as a potential biomarker of AD. This hypothesis requires further confirmation by future long-term cohort studies with large sample size.

In addition, the brain-derived neurotrophic factor (BDNF) gene is a promising risk factor for AD. A common single nucleotide polymorphism (SNP) in the BDNF gene produces Val66Met that affects intracellular packaging and activity-dependent secretion of BDNF and consequently affects human memory function [134]. And serum BNDF levels were significantly increased in MCI and AD patients when compared to healthy subjects, supporting the hypothesis of an upregulation of BDNF in both preclinical phase of dementia (MCI) and clinical stages of AD [135]. Moreover, studies have demonstrated that carriage of the Met allele is associated with poor memory, reduced hippocampal volume, and lower hippocampal activation on functional imaging [136], implying that Met-BDNF polymorphism could be an additional risk factor for rapid disease progression in preclinical AD [137, 138]. The translocase of outer mitochondrial membrane 40 (TOMM40), which is in linkage disequilibrium with APOE, has received increasing attention as a promising gene in AD [139]. In the APOE ε4/BDNF met carriers, a significant inverse relationship existed between episodic memory scores and amyloid, highlighting a potential role of BDNF polymorphisms in the preclinical phase of Aβ deposition [140]. Further studies should be conducted to elucidate whether these gene factors can be considered to be useful prognostic markers of accelerated cognitive decline and hippocampal degeneration in individuals in the preclinical stage of AD.

OTHER POTENTIAL AD-RELATED BIOMARKERS

There are many other clinical or laboratory abnormalities that can potentially serve as biomarkers for the prediction of AD. For example, researchers speculate that event-related brain potentials (ERPs), including primarily of summed excitatory and inhibitory postsynaptic potentials, may provide non-invasive measures of synaptic dysfunction underlying very early cognitive alternations in preclinical familial AD [141]. The P600, one ERP component that are of special interest for investigation of AD, can index both memory encoding and retrieval processes [142]. Recent study suggested that abnormal P600 word repetition effects in cognitively normal elderly persons may be an important sign of synaptic dysfunction and preclinical AD [143]. The well-established sensitivity of ERPs to cognitive processes as well as their practical advantages (e.g., inexpensive, noninvasive, direct measure of summated synaptic currents) supports their potential use as a cost-effective index for early AD. Moreover, specific markers of excitotoxicity, synaptic dysregulation, synapse loss and neuronal loss (including apoptosis) might contribute more-precise staging of the evolution of AD pathology, and longitudinal studies in larger cohorts are necessary to develop techniques to image markers of some of these processes in future.

Numerous studies showed an altered pattern of AβPP expression in platelets of sporadic AD patients compared with either healthy control subjects or patients with non-Alzheimer-type dementia [144–146]. Another recent study illustrates that a new AβPP 115 kDa isoform in platelets can be considered as an additional tool for the development of a reliable diagnostic test to detect preclinical stages of AD [147]. However, its role as a biomarker for the early diagnosis of AD needs to be validated.

Olfactory impairment is a characteristic feature of clinically-established AD which may arise earlier in its evolution [148]. Study using an olfactory 'stress test' (OST), targeting the olfactory bulb, supported that the OST using atropine as an olfactory probe holds promise as a simple, inexpensive screen for preclinical AD [149]. Longitudinal studies, is still needed to verify this burgeoning measure possibility.

In addition, YKL-40 (chitinase-3 like-1, human cartilage glycoprotein-39, and chondrex), a secreted 40-kDa glycoprotein with chitin binding ability but no chitinase activity, found to be significantly more abundant in AD CSF [150]. Reports suggest it is involved in neuroinflammation and tissue remodeling and an upregulation in AD brain [151]. Moreover, the current data demonstrate that YKL-40 combined with $A\beta_{42}$ has potential prognostic utility as a biomarker for preclinical AD [152]. These potential AD-related biomarkers, although interesting and worth pursuing,

require validation before the biomarkers apply to clinical practice.

Increasing studies have showed that visual deficits are common and crippling in AD patients and histopathological alterations found in the retina and brain are similar [153]. Moreover, recent neurophysiological and imaging studies have revealed that changes in visuospatial perception functions can be detected in the early stages of AD [154]. To confirm whether subtle morphological and functional changes in microglial and neuronal activities may also occur in retina during the preclinical stages of AD, numerous studies explore the signs of microglia activation and TNFα induction in the pre-plaque retina of AD mouse models [155]. The results supported that microglial activation is triggered already in the retina of pre-plaque AD mice, which is very relevant in the quest for the earliest AD biomarkers. Meanwhile these alterations can be detected more easily by modern imaging and electrophysiological exploration than those occurring in the hippocampus and therefore, may serve as the earliest diagnostic biomarkers for AD.

TEST COMBINATIONS AND CHALLENGES AHEAD

A combination of tests improved the diagnostic accuracy of predicting cognitive decline in people with MCI from that achieved with either modality alone [92]. The combination of biomarkers from different methodologies is believed to be of incrementally added risk-value to accurately identify asymptomatic and prodromal individuals who will likely progress to AD [156]. For example, the added value of a combination of biomarkers for the prediction of AD has been assessed in several studies, using MRI-volumetric measures in combination with CSF biomarkers [157, 158]. From a clinical point of view, the combination of the executive function and regional FDG-PET provides high sensitivity and specificity for the detection of clinical worsening in elderly cognitive healthy subjects, possibly at an early stage of AD dementia [101]. In addition, increasing studies are focusing on the vascular and metabolic components of non-genetic risk factors which may contribute to the onset of AD. These non-genetic risk factors, including obesity and diabetes [159], hypertension [160], dyslipidemia [161], cigarette smoking [162], lack of education or low education [163] and traumatic head injury [164], have certain value for reference to predict the onset of AD. However, these various factors as well as genetic factors create the challenge, for both policymakers and practicing clinicians, of where to draw the line between those who should and those who should not be diagnosed with preclinical AD. Such a decision is supported by evidence, but current clinical trials have not covered all possible subgroups. Thus it is essential to develop accurate prediction models, and these models should be accessible to clinicians.

Although advances in biomarkers studies have supported detection of preclinical AD, substantial challenges remain to be overcome before their role in prediction of onset of AD can be validated and translated into clinical practice. For example, the biomarkers currently in use fail to reflect the underlying pathophysiological process of AD [165]. Existing biomarkers of amyloid, which primarily indicate when Aβ has begun to accumulate and form fibrillar deposits, might not be sensitive to the soluble Aβ species that are thought to be particularly toxic to synaptic function [166]. Thus, currently available biomarkers are probably tracking disease progression at some level, but clearly much remains to be discovered in this realm. Moreover, it is critical for us to realize that understanding a disease is not to describe what is obvious in greater detail but to present the causes for what we can see and measure [167]. Numerous challenges face translation of group findings into individual use, and ultimately prevention of dementia. However, we still continue to move forward with studies that resolve uncertainties regarding the validity and value of biomarker-defined preclinical stages of AD, especially the quest for an "ideal biomarker". And these "ideal biomarker" should meet following criteria: 1) they should detect a fundamental feature of the molecular pathogenesis or neuropathology of AD; 2) they should be validated in neuropathologically confirmed AD cases; 3) they should have a sensitivity >80% for detecting AD and a specificity >80% for differentiating AD from other dementias; 4) they should be able to detect AD in its early stages; 5) they should be reliable, reproducible, noninvasive, simple to perform, inexpensive and adaptable in routine clinical practice.

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