intended purely for research purposes, and have no clinical or diagnostic utility at the present time. We hope these criteria will enable researchers to characterize further the sequence of biological events over the course of preclinical AD, refine biomarker criteria that will best predict clinical outcome, and ultimately aid in selecting appropriate populations for preclinical therapeutic intervention.

2. Redefining the earliest stages of AD

The term "Alzheimer's disease" has referred in some contexts to the neuropathological criteria for AD and in other contexts to the clinical syndrome of progressive cognitive and behavioral impairment, typically at the stage of AD dementia. As we move toward defining the earliest stages of AD, the dissociation between these two connotations of the term "Alzheimer's disease" becomes particularly salient. It has become increasingly clear that both the underlying pathophysiological process of AD and its clinical symptomatology are best conceptualized as a continuum or a trajectory, and that these processes may evolve in parallel but temporally offset trajectories.

To facilitate the possibility of future presymptomatic/preclinical treatment of AD, our working group, as well as the other two groups, felt it was important *to define AD as encompassing the underlying pathophysiological disease process,* as opposed to having "AD" connote only the clinical stages of the disease [2]. To disambiguate the term "AD," it may be useful to refer to evidence of the underlying brain disease process as AD-pathophysiological process (abbreviated as AD-P) and the clinical phases of the illness as "AD-Clinical" (abbreviated as AD-C), which would include not only AD dementia but also individuals with MCI due to AD-P. AD-P is thought to begin years before the emergence of AD-C. In particular, emerging evidence from both genetic at-risk and aging cohorts suggests that there may be a time lag of a decade or more between the beginning of the pathological cascade of AD and the onset of clinically evident impairment. We postulate that AD begins with a long asymptomatic period during which the pathophysiological process is progressing, and that individuals with biomarker evidence of early AD-P are at increased risk for developing cognitive and behavioral impairment and progression to AD dementia (AD-C). The extent to which biomarkers of AD-P predict a cognitively normal individual's subsequent clinical course remains to be clarified, and we acknowledge that some of these individuals will never manifest clinical symptoms in their lifetime. Thus, it is critical to better define the preclinical stage of AD, to determine the factors that best predict the emergence of clinical impairment and progression to eventual AD dementia, and to reveal the biomarker profile that will identify individuals most likely to benefit from early intervention.

The concept of a preclinical phase of disease should not be too foreign because medical professionals readily acknowledge that cancer can be detected at the stage of "carcinoma *in situ*" and that hypercholesterolemia and atherosclerosis can result in narrowing of coronary arteries that is detectable before myocardial infarction. It is widely acknowledged that symptoms are not necessary to diagnose human disease. Type II diabetes, hypertension, renal insufficiency, and osteoporosis are frequently detected through laboratory tests (i.e., biomarkers), and effective treatment can prevent the emergence of symptoms. Thus, we should be open to the idea that AD could one day be diagnosed preclinically by the presence of biomarker evidence of AD-P, which may eventually guide therapy before the onset of symptoms.

The difficulty in the field of AD is that we have not yet established a firm link between the appearance of any specific biomarker in asymptomatic individuals and the subsequent emergence of clinical symptomatology. If we can, however, definitively determine the risk of developing AD dementia and the temporal course of clinical progression associated with AD-P in individuals without dementia or MCI, we will open a crucial window of opportunity to intervene with disease-modifying therapy. Although we hypothesize that the current earliest detectable pathological change will be in the form of Aβ accumulation, it is possible that Aβ accumulation is necessary but not sufficient to produce the clinical manifestations of AD. It is likely that cognitive decline would occur only in the setting of Aβ accumulation plus synaptic dysfunction and/or neurodegeneration, including paired helical filament tau formation and neuronal loss. It also remains unknown whether there is a specific threshold or regional distribution of AD pathology, and/or a specific combination of biomarker abnormalities that will best predict the emergence of clinical symptoms. Evidence also suggests that additional factors, such as brain and cognitive reserve, and conversely, the presence of other age-related brain diseases, may modulate the relationship between AD-P and AD-C. We also recognize that some individuals can evidence all of the diagnostic neuropathological features of AD at autopsy but never express dementia during their life; it remains unknown whether these individuals would have manifested clinical symptoms should they have lived longer. It is also possible that some individuals are relatively resistant to AD-P because of cognitive or brain reserve, protective genetic factors, or environmental influences. Recent advances in antemortem biomarkers now allow us to test the hypothesis that many individuals with laboratory evidence of AD-P are indeed in the preclinical stages of AD, and determine which biomarker and cognitive profiles are most predictive of subsequent clinical decline and emergence of AD-C.

3. The continuum of AD

The other two working groups established by the National Institute on Aging/Alzheimer's Association are focused on developing diagnostic criteria for the clinical stages of MCI and dementia due to underlying AD-P [3–5]. Our group focused on developing *research recommendations*

for the study of individuals who have evidence of early AD pathological changes but do not meet clinical criteria for MCI or dementia. It is likely that even this preclinical stage of the disease represents a continuum from completely asymptomatic individuals with biomarker evidence suggestive of AD-P at risk for progression to AD dementia to biomarker-positive individuals who are already demonstrating very subtle decline but not yet meeting standardized criteria for MCI (refer to accompanying MCI workgroup recommendations by Albert et al). This latter group of individuals might be classified as "Not normal, not MCI" but would be included under the rubric of preclinical AD (Fig. 1). Importantly, this continuum of preclinical AD would also encompass (1) individuals who carry one or more apolipoprotein E (*APOE*) ε4 alleles who are known to have an increased risk of developing AD dementia, *at the point they are AD-P biomarker-positive*, and (2) carriers of autosomal dominant mutations, who are in the presymptomatic biomarker-positive stage of their illness, and who will almost certainly manifest clinical symptoms and progress to dementia.

Our group carefully considered several monikers to best capture this stage of the disease, including "asymptomatic," "presymptomatic," "latent," "premanifest," and "preclinical." The term "preclinical" was felt to best encompass this conceptual phase of the disease process but is not meant to imply that all individuals who have evidence of early AD pathology will necessarily progress to clinical AD dementia. Individuals who are biomarker positive but cognitively normal might currently be defined as "asymptomatic at risk for AD dementia." Indeed, our goal is to better define the factors which best predict cognitive decline in biomarker-positive individuals, so as to move toward an accurate profile of preclinical AD.

4. Models of the pathophysiological sequence of AD

To facilitate the discussion of the concept of a preclinical stage of AD, we propose a theoretical model of the pathophysiological cascade of AD (Fig. 2). It is important to acknowledge that this model, although based on the prevailing evidence, may be incorrect, is certainly incomplete, and will evolve as additional laboratory and clinical studies are completed. Indeed, this model should be viewed as an initial attempt to bring together multiple areas of research into our best estimate of a more coherent whole.

The proposed model of AD views Aβ peptide accumulation as a key early event in the pathophysiological process of AD. However, we acknowledge that the etiology of AD remains uncertain, and some investigators have proposed that synaptic, mitochondrial, metabolic, inflammatory, neuronal, cytoskeletal, and other age-related alterations may play an even earlier, or more central, role than Aβ peptides in the pathogenesis of AD [6,7]. There also remains significant debate in the field as to whether abnormal processing versus clearance of $A\beta_{42}$ is the etiologic event

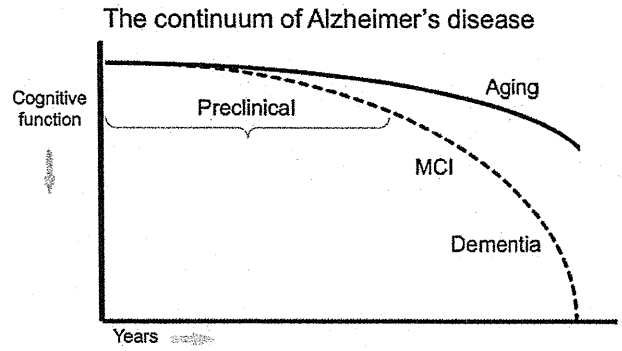

Fig. 1. Model of the clinical trajectory of Alzheimer's disease (AD). The stage of preclinical AD precedes mild cognitive impairment (MCI) and encompasses the spectrum of presymptomatic autosomal dominant mutation carriers, asymptomatic biomarker-positive older individuals at risk for progression to MCI due to AD and AD dementia, as well as biomarker-positive individuals who have demonstrated subtle decline from their own baseline that exceeds that expected in typical aging, but would not yet meet criteria for MCI. Note that this diagram represents a hypothetical model for the pathological-clinical continuum of AD but does not imply that all individuals with biomarker evidence of AD-pathophysiological process will progress to the clinical phases of the illness.

in sporadic, late-onset AD [8]. Some investigators have suggested that sequestration of Aβ into fibrillar forms may even serve as a protective mechanism against oligomeric species, which may be the more synaptotoxic forms of Aβ [9–11]. However, of all the known autosomal dominant, early onset forms of AD are thought to be, at least in part, due to alterations in amyloid precursor protein (*APP*) production or cleavage. Similarly, trisomy 21 invariably

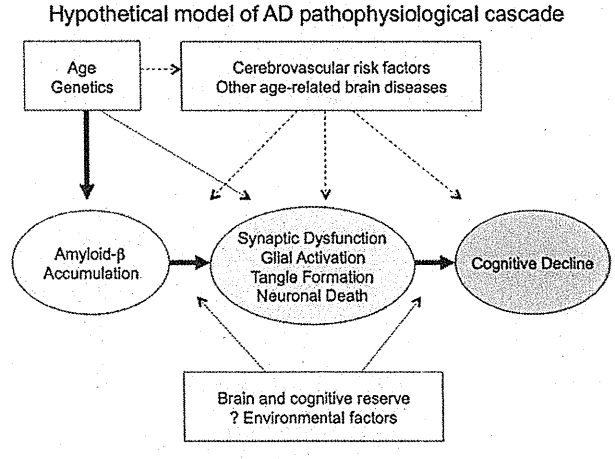

Fig. 2. Hypothetical model of the Alzheimer's disease (AD) pathophysiological sequence leading to cognitive impairment. This model postulates that amyloid beta (Aβ) accumulation is an "upstream" event in the cascade that is associated with "downstream" synaptic dysfunction, neurodegeneration, and eventual neuronal loss. Note that although recent work from animal models suggests that specific forms of Aβ may cause both functional and morphological synaptic changes, it remains unknown whether Aβ is sufficient to incite the neurodegenerative process in sporadic late-onset AD. Age and genetics, as well as other specific host factors, such as brain and cognitive reserve, or other brain diseases may influence the response to Aβ and/or the pace of progression toward the clinical manifestations of AD.

results in AD-P in individuals who have three intact copies of the APP coding region located on chromosome 21. Finally, *APOE*, the major genetic risk factor for late-onset AD, has been implicated in amyloid trafficking and plaque clearance. Both autopsy and biomarker studies (see later in the text) similarly suggest that $A\beta_{42}$ accumulation increases with advanced aging, the greatest risk factor for developing AD. At this point, it remains unclear whether it is meaningful or feasible to make the distinction between Aβ as a risk factor for developing the clinical syndrome of AD versus Aβ accumulation as an early detectable stage of AD because current evidence suggests that both concepts are plausible.

Also, it is clear that synaptic depletion, intracellular hyperphosphorylated forms of tau, and neuronal loss invariably occur in AD, and at autopsy, these markers seem to correlate better than plaque counts or total Aβ load with clinical impairment. Although we present evidence later that the presence of markers of "upstream" Aβ accumulation is associated with markers of "downstream" pathological change, including abnormal tau, neural dysfunction, glial activation, and neuronal loss and atrophy, it remains to be proven that Aβ accumulation is sufficient to incite the downstream pathological cascade of AD. It remains unknown whether this neurodegenerative process could be related to direct synaptic toxicity due to oligomeric forms of Aβ, disruption of axonal trajectories from fibrillar forms of Aβ, or a "second hit" that results in synaptic dysfunction, neurodegeneration, neurofibrillary tangle formation, and eventually neuronal loss.

Epidemiological data suggest there are significant modulating factors that may alter the pace of the clinical expression of AD-P, although evidence that these factors alter the underlying pathophysiological process itself is less secure. Large cohort studies have implicated multiple health factors that may increase the risk for developing cognitive decline and dementia thought to be caused by AD [12]. In particular, vascular risk factors such as hypertension, hypercholesterolemia, and diabetes have been associated with an increased risk of dementia, and may contribute directly to the effect of AD pathology on the aging brain [13,14]. Depressive symptomatology, apathy, and chronic psychological distress have also been linked to increased risk of manifesting MCI and dementia [15–17]. It also remains unclear whether there are specific environmental exposures, such as head trauma, that may influence the progression of the pathophysiological sequence or the clinical expression of the pathology. On the positive side, there is some evidence that engagement in specific activities, including cognitive, physical, leisure, and social activity, may be associated with decreased risk of MCI and AD dementia [18].

The temporal lag between the appearance of AD-P and the emergence of AD-C also may be altered by factors such as brain or cognitive reserve [19]. The concept of reserve was originally invoked to provide an explanation for the observation that the extent of AD histopathological changes at autopsy did not always align with the degree of clinical impairment, and can be thought of as the ability to tolerate higher levels of brain injury without exhibiting clinical symptoms. "Brain reserve" refers to the capacity of the brain to withstand pathological insult, perhaps because of greater synaptic density or larger number of healthy neurons, such that sufficient neural substrate remains to support normal function. In contrast, "cognitive reserve" is thought to represent the ability to engage alternate brain networks or cognitive strategies to cope with the effects of encroaching pathology. It is not clear, however, that the data support a sharp demarcation between these two constructs because many factors, such as higher socioeconomic status or engagement in cognitively stimulating activities, may contribute to both forms of reserve. Higher education and socioeconomic status have been associated with lower age-adjusted incidence of AD diagnosis. Recent studies suggest that high reserve may primarily influence the capability of individuals to tolerate their AD-P for longer periods, but may also be associated with rapid decline after a "tipping point" is reached and compensatory mechanisms begin to fail [20,21].

5. Biomarker model of the preclinical stage of AD

A biomarker model has been recently proposed in which the most widely validated biomarkers of AD-P become abnormal and likewise reach a ceiling in an ordered manner [22]. This biomarker model parallels the hypothetical pathophysiological sequence of AD discussed previously, and is particularly relevant to tracking the preclinical stages of AD (Fig. 3). Biomarkers of brain Aβ amyloidosis include reductions in CSF $A\beta_{42}$ and increased amyloid tracer retention on positron emission tomography (PET) imaging. Elevated CSF tau is not specific to AD and is thought to be a biomarker of neuronal injury. Decreased fluorodeoxyglucose 18F (FDG) uptake on PET with a temporoparietal pattern of hypometabolism is a biomarker of AD-related synaptic dysfunction. Brain atrophy on structural magnetic resonance imaging (MRI) in a characteristic pattern involving the medial temporal lobes, paralimbic and temporoparietal cortices is a biomarker of AD-related neurodegeneration.

This biomarker model was adapted from the original graph proposed by Jack et al [22] to expand the preclinical phase, and has the following features: (1) Aβ accumulation biomarkers become abnormal first and a substantial Aβ load accumulates before the appearance of clinical symptoms. The lag phase between Aβ accumulation and clinical symptoms remains to be quantified, but current theories suggest that the lag may be for more than a decade. Similar to the hypothetical pathophysiological model described previously, interindividual differences in this time lag are likely caused by differences in brain reserve, cognitive reserve, and the added contributions of coexisting pathologies. Note that in this biomarker model, brain Aβ accumulation is *necessary but not sufficient* to produce the clinical symptoms of MCI and dementia, (2) biomarkers of synaptic dysfunction, including FDG and functional MRI (fMRI), may demonstrate abnormalities very early, particularly in *APOE* gene

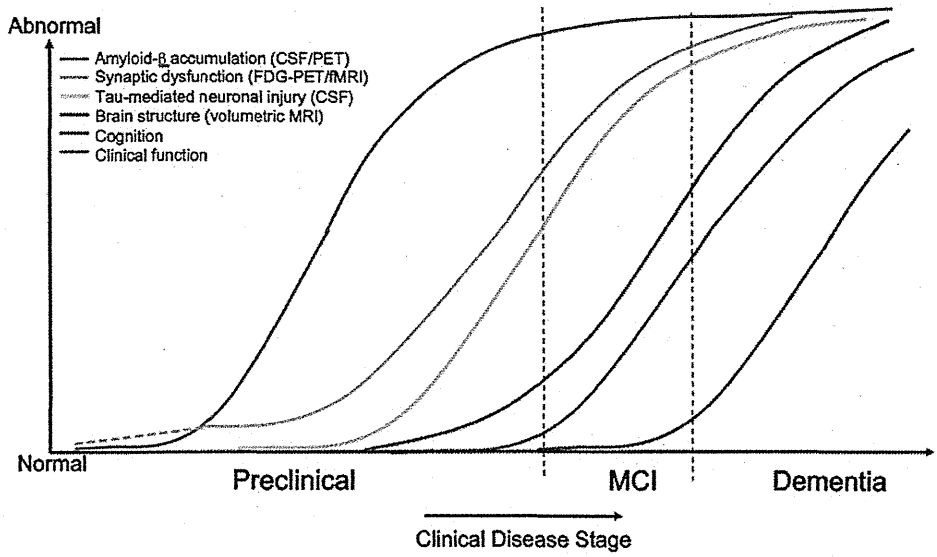

Fig. 3. Hypothetical model of dynamic biomarkers of the AD expanded to explicate the preclinical phase: Aβ as identified by cerebrospinal fluid $A\beta_{42}$ assay or PET amyloid imaging. Synaptic dysfunction evidenced by fluorodeoxyglucose (F18) positron emission tomography (FDG-PET) or functional magnetic resonance imaging (fMRI), with a dashed line to indicate that synaptic dysfunction may be detectable in carriers of the ε4 allele of the apolipoprotein E gene before detectable Aβ deposition. Neuronal injury is evidenced by cerebrospinal fluid tau or phospho-tau, brain structure is evidenced by structural magnetic resonance imaging. Biomarkers change from normal to maximally abnormal (*y*-axis) as a function of disease stage (*x*-axis). The temporal trajectory of two key indicators used to stage the disease clinically, cognitive and behavioral measures, and clinical function are also illustrated. Figure adapted with permission from Jack et al [22].

ε4 allele carriers, who may manifest functional abnormalities before detectable Aβ deposition [23–25]. The severity and change over time in these synaptic markers correlate with clinical symptoms during MCI and AD dementia, (3) structural MRI is thought to become abnormal a bit later, as a marker of neuronal loss, and MRI retains a close relationship with cognitive performance through the clinical phases of MCI and dementia [26], (4) none of the biomarkers is static; rates of change in each biomarker change over time and follow a nonlinear time course, which is hypothesized to be sigmoid shaped, and (5) anatomic information from imaging biomarkers provides useful disease staging information in that the topography of disease-related imaging abnormalities changes in a characteristic manner with disease progression.

6. Biomarker and autopsy evidence linking AD pathology to early symptomatology

Several multicenter biomarker initiatives, including the Alzheimer's Disease Neuroimaging Initiative; the Australian Imaging, Biomarkers and Lifestyle Flagship Study of Aging; as well as major biomarker studies in preclinical populations at several academic centers, are ongoing. These studies have already provided preliminary evidence that biomarker abnormalities consistent with AD pathophysiological process are detectable before the emergence of overt clinical symptomatology and are predictive of subsequent cognitive decline. Many of the recent studies have focused on markers of Aβ using either CSF assays of $A\beta_{42}$ or PET amyloid imaging with radioactive tracers that bind to fibrillar forms of Aβ. Both CSF and PET amyloid imaging studies suggest that a substantial proportion of clinically normal older individuals demonstrate evidence of Aβ accumulation [27–32]. The exact proportion of "amyloid-positive" normal individuals is dependent on the age and genetic background of the cohort, but ranges from approximately 20% to 40% and is very consonant with large postmortem series [33,34]. Furthermore, there is evidence that the AD-P detected at autopsy is related to episodic memory performance even within the "normal" range [35]. Interestingly, the percentage of "amyloid-positive" normal individuals at autopsy detected at a given age closely parallels the percentage of individuals diagnosed with AD dementia a decade later [36,37] (Fig. 4). Similarly, genetic at-risk cohorts demonstrate evidence of Aβ accumulation many years before detectable cognitive impairment [38–41]. These data support the hypothesis that there is a lengthy temporal lag between the appearance of detectable AD-P and the emergence of AD-C.

Multiple groups have now reported that cognitively normal older individuals with low CSF $A\beta_{1-42}$ or high PET amyloid binding demonstrate disruption of functional networks [42–44] and decreased brain volume [45–49], consistent with the patterns seen in AD. There have been variable reports in the previously published data thus far, regarding whether Aβ-positive individuals demonstrate lower neuropsychological test scores at the time of biomarker study [50–54], which may represent heterogeneity in where these individuals fall on the preclinical continuum, the cognitive measures evaluated, and the degree of cognitive

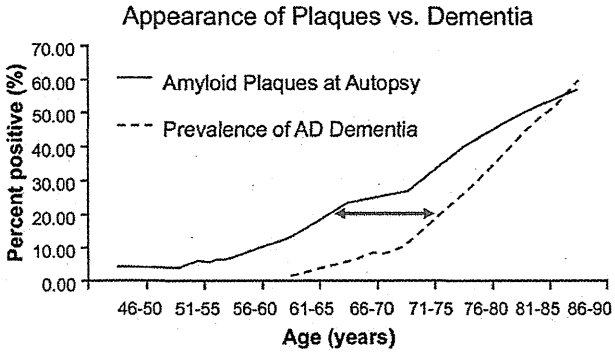

Fig. 4. Postulated temporal lag of approximately a decade between the deposition of Aβ (% of individuals with amyloid plaques in a large autopsy series [68]) and the clinical syndrome of AD dementia (estimated prevalence from three epidemiological studies [69–71]). Figure courtesy of Mark Mintun and John Morris, Washington University.

reserve in the cohorts. A few early studies have reported that Aβ positivity in clinically normal older individuals is associated with an increased rate of atrophy [55] and an increased risk of cognitive decline and progression to dementia [56–62]. Multiple studies focused on other biomarkers, including volumetric MRI, FDG-PET, or plasma biomarkers, in cohorts of clinically normal older individuals have also reported evidence that these markers are predictive of cognitive decline (refer [63,64] for recent examples). Additional longitudinal studies are clearly needed to confirm these findings and to elucidate the combination of factors that best predict likelihood and rate of decline, and to better understand individual diff-erences in risk for decline.

As a complement to longitudinal studies in the population at risk by virtue of age, researchers continue to detect and track the biological and cognitive changes associated with the predisposition to AD in cognitively normal people at differential genetic risk for AD alone or in conjunction with other risk factors (such as a person's reported family history of the disease). To date, the best established genetic risk factors for AD include common allelic variants of *APOE*; the major late-onset AD susceptibility gene; uncommon early-onset AD-causing mutations in the presenilin 1, presenilin 2, and APP genes; and trisomy 21 (Down syndrome). Biomarker studies in presymptomatic carriers of these genetic risk factors have revealed evidence of Aβ accumulation on CSF and PET amyloid imaging, as well as FDG-PET hypometabolism, fMRI abnormalities, and brain atrophy that may precede symptoms by more than a decade.

7. Cognitive studies

Despite the clear potential of biomarkers for detecting evidence of the AD pathophysiological process, it is important not to lose sight of the potential that behavioral markers hold for early identification. Tests developed by both neuropsychological and cognitive aging researchers have provided evidence that normal aging is accompanied by declines in speed of information processing, executive function (working memory, task switching, inhibitory function), and reasoning. Studies that have conducted assessments of cognitive function at multiple time points before dementia have also shown consistently a long period of gradual cognitive decline in episodic memory as well as nonmemory domains progressing up to a decade before onset of dementia. Importantly, in studies that have modeled the curve of cognitive change versus time, the preclinical trajectory suggests not only a long- and slow rate of presymptomatic change but also a period of acceleration of performance decrement that may begin several years before MCI onset [65]. Recent studies also suggest that self-report of subtle cognitive decline, even in the absence of significant objective impairment on testing, may portend future decline in older individuals. Despite the existence of multiple studies spanning thousands of participants, the promise of both subjective and objective cognitive measures for assessing risk of progression to AD in individual elders has not yet been fully realized. It is likely that measured change in cognition over time will be more sensitive than any one-time measure. Additional longitudinal studies of older individuals, perhaps combining biomarkers with measures sensitive to detecting very subtle cognitive decline, are clearly needed.

8. Caveats

Although the aforementioned studies provide compelling evidence that markers of Aβ in "normal" older individuals are associated with other brain alterations consistent those seen in AD dementia, and that specific factors may

Table 1
Staging categories for preclinical AD research

Stage	Description	Aβ (PET or CSF)	Markers of neuronal injury (tau, FDG, sMRI)	Evidence of subtle cognitive change
Stage 1	Asymptomatic cerebral amyloidosis	Positive	Negative	Negative
Stage 2	Asymptomatic amyloidosis + "downstream" neurodegeneration	Positive	Positive	Negative
Stage 3	Amyloidosis + neuronal injury + subtle cognitive/behavioral decline	Positive	Positive	Positive

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid beta; PET, positron emission tomography; CSF, cerebrospinal fluid; FDG, fluorodeoxyglucose (18F); sMRI, structural magnetic resonance imaging.

accurately predict those individuals who are at a higher risk of progression to AD-C, it is important to note several potential confounding issues in the majority of these studies. It is likely that many of these studies suffer from cohort biases. In particular, the biomarker and cognitive studies likely are not representative of the general older population because they are typically "samples of convenience," that is, volunteer cohorts who tend to come from highly educated and socioeconomic status backgrounds. These individuals may also be less likely to harbor typical age-related comorbidities that may influence the rate of cognitive decline. Older individuals who are willing to participate in such intensive studies may also represent the "volunteer gene," and may be more actively engaged than the typical aging population. Conversely, these cohorts may include individuals who self-select for this research because of subjective concerns about their own memory function or positive family history, as reflected by the high rate of *APOE* ε4 carriers in some of these cohorts.

It is also important to note that although these biomarkers have revolutionized the field of early AD, these markers are merely "proxies" for the underlying disease and may not fully reflect the biological processes in the living brain. For example, both CSF and PET amyloid imaging markers seem to be estimates of the deposition of fibrillar forms of Aβ, and may not provide information about oligomeric forms, which may be the relevant species for synaptic toxicity. Similarly, our proxy measurements for synaptic dysfunction, such as fMRI or FDG-PET, are indirect measurements of neural function. Other markers of neurodegeneration such as CSF tau and volumetric MRI are not specific to the AD process. Finally, it is important to acknowledge that the relationship between biomarkers and cognition may vary significantly across age and genetic cohorts. In particular, the dissociation between the presence or absence of AD-P and clinical symptomatology in the oldest-old needs to be better understood.

Finally, it is important to re-emphasize that although Aβ deposition and neuritic plaque formation are required for the diagnosis of definite AD, and that current evidence suggests that Aβ accumulation is an early detectable stage of the pathological-clinical continuum of AD, the role of Aβ as the etiologic agent in sporadic late-onset AD remains to be proven. There may be pathophysiological events that are "upstream" of Aβ accumulation yet to be discovered, and the relationship between Aβ and neurodegeneration is not yet clear. In particular, the failure of biologically active Aβ-lowering therapies to demonstrate clinical benefit thus far is of concern. Thus, it is important to continue research in alternative pathophysiological pathways and therapeutic avenues.

9. Draft operational research framework for staging preclinical AD

To facilitate future studies, we propose draft operational research criteria to define study cohorts at risk for developing AD dementia for use in (1) longitudinal natural history studies to determine whether the presence of Aβ markers, either in isolation or in combination with additional markers of neurodegeneration, is predictive of cognitive decline in clinically normal older individuals, and (2) clinical trials of potential disease-modifying agents to investigate effects on biomarker progression and/or the emergence of clinical symptoms.

We emphasize again that this framework is not intended to serve as diagnostic criteria for clinical purposes. Use of these biomarkers in the clinical setting is currently unwarranted because many individuals who satisfy the proposed research criteria may not develop the clinical features of AD in their lifetime. Inappropriate use of this information in this context could be associated with unwarranted concern because there is currently insufficient information to relate preclinical biomarker evidence of AD to subsequent rates of clinical progression with any certainty.

These research criteria are based on the postulate that AD is characterized by a sequence of biological events that begins far in advance of clinical dementia. On the basis of current evidence from both genetic at-risk and older cohort studies, we put forth the hypothesis that Aβ accumulation, or the stage of cerebral amyloidosis, is currently one of the earliest measurable stages of AD, and occurs before any other evidence of cognitive symptomatology. We postulate that the presence of biomarker "positivity" for Aβ in clinically normal older individuals, particularly in combination with evidence of abnormality on other biomarkers of AD-P, may have implications for the subsequent course of AD-C and the responsiveness to treatments targeting AD-P.

Recognizing that the preclinical stages of AD represent a continuum, including individuals who may never progress beyond the stage of Aβ accumulation, we further suggest the following staging schema (see Table 1), which may prove useful in defining research cohorts to test specific hypotheses. Research cohorts could be selected on the basis of these staging criteria, to optimize the ability to ascertain the specific outcomes important for a given type (e.g., natural history or treatment trial) and duration of the study. Evidence of "downstream" biomarkers or subtle cognitive symptoms in addition to evidence of Aβ accumulation may increase the likelihood of rapid emergence of cognitive symptomatology and clinical decline to MCI within several years. The presence of one or more of these additional biomarkers would indicate that individuals are already experiencing early neurodegeneration, and as such, it is possible that amyloid-modifying therapies may be less efficacious after the downstream pathological process is set in motion. There are specific circumstances, however, such as pharmaceutical industry trials that may require a cognitive or clinical endpoint, rather than relying solely on biomarker outcomes. In these cases, it may be advantageous to enrich the study population with individuals in late preclinical stages of AD with evidence of very subtle cognitive change, who would be most likely to rapidly decline and manifest MCI within a short period (see Fig. 5). We recognize that these stages

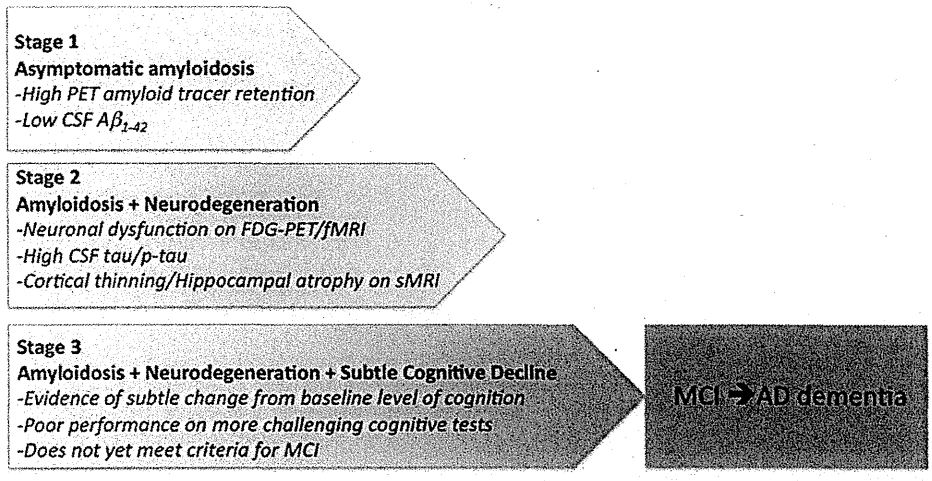

Fig. 5. Graphic representation of the proposed staging framework for preclinical AD. Note that some individuals will not progress beyond Stage 1 or Stage 2. Individuals in Stage 3 are postulated to be more likely to progress to MCI and AD dementia. Abbreviations: AD, Alzheimer's disease; Ab, amyloid beta; PET, position emission tomography; CSF, cerebrospinal fluid; FDG, fluorodeoxyglucose, fMRI, functional magnetic resonance imaging, sMRI, structural magnetic resonance imaging.

will likely require further modification as new findings emerge, and that the feasibility of delineating these stages in recruiting clinical research cohorts remains unclear. It may be easiest to recruit individuals on the basis of Aβ positivity and perform post hoc analyses to determine the predictive value of specific combinations of biomarker abnormalities. These proposed research criteria are intended to facilitate the standardized collection of new data to better define the spectrum of preclinical AD, and to elucidate the endophenotype of individuals who are most likely to progress toward AD-C.

9.1. Stage 1: The stage of asymptomatic cerebral amyloidosis

These individuals have biomarker evidence of Aβ accumulation with elevated tracer retention on PET amyloid imaging and/or low $A\beta_{42}$ in CSF assay, but no detectable evidence of additional brain alterations suggestive of neurodegeneration or subtle cognitive and/or behavioral symptomatology. The standards for determining "amyloid-positivity" are still evolving (refer to the next section). Although recent work suggests there may be a CSF $A\beta_{42}$ cutoff value that is predictive of progression from MCI to AD dementia [66], it is unknown whether a similar threshold will be optimal in prediction of decline in individuals with normal or near normal cognition. Similarly, using PET imaging techniques, it remains unknown whether a summary numeric threshold within an aggregate cortical region or within specific anatomic region will provide the most useful predictive value. Recent data suggest that although CSF $A\beta_{42}$ is strongly inversely correlated with quantitative PET amyloid imaging measures (distribution value ratio or standardized uptake value), there are some individuals who demonstrate decreased CSF $A\beta_{42}$ and who would not be considered amyloid positive on PET scans [67]. It remains unclear whether this finding reflects different thresholds used across these techniques or if decreased CSF $A\beta_{42}$ is an earlier marker of accumulation. In addition, there may be genetic effects that are specific to CSF or PET markers of Aβ.

As mentioned previously, we note that the currently available CSF and PET imaging biomarkers of Aβ primarily provide evidence of amyloid accumulation and deposition of fibrillar forms of amyloid. Although limited, current data suggest that soluble or oligomeric forms of Aβ are likely in equilibrium with plaques, which may serve as reservoirs, but it remains unknown whether there is an identifiable preplaque stage in which only soluble forms of Aβ are present. Because laboratory data increasingly suggest that oligomeric forms of amyloid may be critical in the pathological cascade, there is ongoing work to develop CSF and plasma assays for oligomeric forms of Aβ. There are also emerging data from genetic-risk cohorts that suggest early synaptic changes may be present before evidence of amyloid accumulation using currently available amyloid markers. Thus, it may be possible in the future to detect a stage of disease that precedes stage 1.

9.2. Stage 2: Amyloid positivity + evidence of synaptic dysfunction and/or early neurodegeneration

These individuals have evidence of amyloid positivity and presence of one or more markers of "downstream" AD-P-related neuronal injury. The current markers of neuronal injury with the greatest validation are: (1) elevated CSF tau or phospho-tau, (2) hypometabolism in an AD-like pattern (i.e., posterior cingulate, precuneus, and/or temporoparietal cortices) on FDG-PET, and (3) cortical thinning/gray matter loss in a specific anatomic distribution (i.e., lateral

and medial parietal, posterior cingulate, and lateral temporal cortices) and/or hippocampal atrophy on volumetric MRI. Future markers may also include fMRI measures of default network connectivity. Although previous studies have demonstrated that, on average, amyloid-positive individuals demonstrate significantly greater abnormalities on these markers as compared with amyloid-negative individuals, there is significant interindividual variability. We hypothesize that amyloid-positive individuals with evidence of early neurodegeneration may be farther down the trajectory (i.e., in later stages of preclinical AD). It remains unclear whether it will be feasible to detect differences among these other biomarkers of AD-P, but there is some evidence that early synaptic dysfunction, as assessed by functional imaging techniques such as FDG-PET and fMRI, may be detectable before volumetric loss.

9.3. Stage 3: Amyloid positivity + evidence of neurodegeneration + subtle cognitive decline

We postulate that individuals with biomarker evidence of amyloid accumulation, early neurodegeneration, and evidence of subtle cognitive decline are in the last stage of preclinical AD, and are approaching the border zone with the proposed clinical criteria for MCI. These individuals may demonstrate evidence of decline from their own baseline (particularly if proxies of cognitive reserve are taken into consideration), even if they still perform within the "normal" range on standard cognitive measures. There is emerging evidence that more sensitive cognitive measures, particularly with challenging episodic memory measures, may detect very subtle cognitive impairment in amyloid-positive individuals. It remains unclear whether self-complaint of memory decline or other subtle neurobehavioral changes will be a useful predictor of progression, but it is possible that the combination of biomarkers and subjective assessment of subtle change will prove to be useful.

10. Need for additional study

We propose a general framework with biomarker criteria for the study of the preclinical phase of AD; however, more work is needed to clarify the optimal CSF assays, PET or MRI analytic techniques, and in particular, the specific thresholds needed to meet these criteria. There are significant challenges in implementing standardized biomarker "cut-off" values across centers, studies, and countries. Work to standardize and validate both fluid-based and imaging biomarker thresholds is ongoing in multiple academic and pharmaceutical industry laboratories, as well as in several multicenter initiatives. These criteria will need to be validated in large multicenter natural history studies, or as provisional criteria for the planning of preventative clinical trials. For instance, it will be important to establish the test–retest and cross-center reliability of biomarker measurements, further characterize the sequence of biomarker changes, and the extent to which these biomarkers predict subsequent clinical decline or clinical benefit. In particular, there is an important need to evaluate methods for determining "amyloid-positivity" because it remains unclear whether there is a biologically relevant continuum of Aβ accumulation, or whether there is a clear threshold or "cut-off" value that could be defined on the basis of predictive value for subsequent clinical decline, as has been suggested in several CSF studies [28,66]. It also remains unknown whether these thresholds should be adjusted for age or genotype. After these thresholds are established, it may be most feasible to select research cohorts for large studies solely on the basis of "amyloid-positivity" on CSF or PET amyloid imaging, and to use additional biomarker and cognitive measures for post hoc analyses to determine additional predictive value.

Although recent advances in biomarkers have revolutionized our ability to detect evidence of early AD-P there is still a need for novel biomarker development. In particular, although the current biomarkers provide evidence of Aβ deposition, an *in vivo* marker of oligomeric forms of Aβ would be of great value. Imaging markers of intraneuronal pathology, including specific markers of specific forms of tau/tangles and alpha-synuclein, are also needed. In addition, more sensitive imaging biomarkers that can detect early synaptic dysfunction and functional and structural disconnection, such as fMRI and diffusion tensor imaging, may one day prove to be useful to track early response to amyloid-lowering therapies. Finally, we may be able to use the currently available biomarkers as a new "gold standard" to re-evaluate simple blood and urine markers that were discarded on the basis of excessive overlap between clinically normal and AD patients. The significant proportion of clinically normal individuals who are "amyloid-positive" on both CSF and PET imaging may have confounded previous studies attempting to differentiate "normal" controls from patients with AD.

Similarly, additional work is required to identify and validate neuropsychological and neurobehavioral measures to detect the earliest clinical manifestations of AD. We need to develop sensitive measures in multiple cognitive and behavioral domains that will reveal evidence of early synaptic dysfunction in neural networks vulnerable to AD pathology. We also need to develop measures of very early functional changes in other domains, including social interaction, mood, psychomotor aspects of function, and decision making. These measures would allow us to link better the pathological processes to the emergence of clinical symptoms, and may be particularly useful to monitor response to potential disease-modifying therapies in these very early stages.

The proposed criteria apply primarily to individuals at risk by virtue of advanced age because inclusion criteria for trials in autosomal dominant mutation carriers and homozygous *APOE* ε4 carriers will be likely defined primarily on genetic status. Trials in genetic-risk populations might use these criteria to stage individuals within the preclinical phase of AD. In genetic-risk cohorts, it may even be possible to detect an even

earlier stage of presymptomatic AD, before the point when there is already detectable cerebral amyloidosis. Several FDG-PET and fMRI studies have suggested that evidence of synaptic dysfunction may be present in young and middle-aged *APOE* ε4 carriers (see Fig. 3), and there may be other biological alterations that are present before significant deposition of fibrillar forms of amyloid that would be preferentially responsive to presymptomatic intervention.

The emerging concept of preclinical AD and the role of biomarkers in the detection and tracking in this stage of the disease have important implications for the development of effective treatments. Therapies for preclinical AD would be intended to postpone, reduce the risk of, or completely prevent the clinical stages of the disorder. As recently noted, the use of clinical endpoints in clinical trials of such treatments would require large numbers of healthy volunteers, large amounts of money, and many years of study. Researchers have raised the possibility of evaluating biomarker endpoints for these treatments in cognitively normal people at increased risk for AD because these studies might be performed more rapidly than otherwise possible. Subjects enrolled in these studies could include individuals with autosomal dominant mutation carriers (with essentially a 100% chance of developing clinical AD) or those at increased risk of developing sporadic AD (e.g., *APOE* ε4 carriers or subjects with biomarker evidence of preclinical AD pathology). The use of biomarkers rather than clinical outcomes could accelerate progress in these trials; however, regulatory agencies must be assured that a given biomarker is "reasonably likely" to predict a clinically meaningful outcome before they would grant approval for treatments tested in trials using biomarkers as surrogate endpoints. Research strategies have been proposed to provide this evidence by embedding the most promising biomarkers in preclinical AD trials of people at the highest imminent risk of clinical onset to establish a link between a biomarker effect and the onset of clinical symptoms of AD. We envision the time when the scientific means and accelerated regulatory approval pathway support multiple preclinical AD trials using biomarkers to identify subjects and provide shorter term outcomes, such that demonstrably effective treatments to ward off the clinical stages of AD are found as quickly as possible. There are several burgeoning efforts to design and conduct clinical trials in both genetic at-risk and amyloid-positive older individuals, including the Dominantly Inherited Alzheimer Network (study of familial AD), the Alzheimer Prevention Initiative, and Anti-Amyloid Treatment in Asymptomatic AD (A4) trial being considered by the Alzheimer's Disease Cooperative Study.

Finally, the ethical and practical implications surrounding the issues of future implementation of making a "diagnosis" of AD at a preclinical stage need to be studied, should the postulates put forth previously prove to be correct. Although at this point our recommendations are *strictly for research purposes only*, the public controversy surrounding the identification of asymptomatic individuals with evidence of AD-P raised several important points that the field must consider. In particular, the poignant question of "why would anyone want to know they have AD a decade before they might develop symptoms, if there is nothing they can do about it?" should be carefully considered well before any results from research is translated into clinical practice. First, there may be important reasons, including social and financial planning, why some individuals would want to know their likelihood of developing AD dementia within the next decade, even in the absence of an available disease-modifying therapy. It is our hope, however, that the advances in preclinical detection of AD-P will enable earlier, more effective treatment, just as nearly all of therapeutic gains in cancer, cardiovascular disease, osteoporosis, and diabetes involve treatment before significant clinical symptoms are present. It is entirely possible that promising drugs, particularly amyloid-modifying agents, will fail to affect the clinical course of AD at the stage of dementia or even MCI, when the neurodegenerative process is well entrenched, but may be efficacious at the earliest stages of the AD-P, before the onset of symptoms.

The definitive studies to determine whether the majority of asymptomatic individuals with evidence of AD-P are indeed destined to develop AD dementia, to elucidate the biomarker and/or cognitive endophenotype that is most predictive of cognitive decline, and to determine whether intervention with potential disease-modifying therapies in the preclinical stages of AD will prevent dementia are likely to take more than a decade to fully accomplish. Thus, we must move quickly to test the postulates put forth previously, and adjust our models and study designs as new data become available. Because potential biologically active treatments may be associated with small but significant risk of adverse side effects, we will need to determine whether we can predict the emergence of cognitive symptoms with sufficient certainty to appropriately weigh the risk/benefit ratios to begin treatment in asymptomatic individuals. It is clear that many questions remain to be answered, and that there may be additional factors which will influence the probability of developing clinical AD. However, the considerable progress made over the past two decades now enables a strategic path forward to test these hypotheses, move the field toward earlier intervention, and ultimately, toward the prevention of AD dementia.

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References

[1] Morris JC. Early-stage and preclinical Alzheimer disease. Alzheimer Dis Assoc Disord 2005;19:163–5.

[2] Dubois B, Feldman HH, Jacova C, Cummings JL, Dekosky ST, Barberger-Gateau P, et al. Revising the definition of Alzheimer's disease: a new lexicon. Lancet Neurol 2010;9:1118–27.

[3] Jack CR Jr, Albert MS, Knopman DS, McKhann GM, Sperling RA, Carrillo MC, et al. Introduction to the recommendations from the National Institute on Aging–Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:257–62.

[4] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging–Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:270–9.

[5] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging–Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:263–9.

[6] Pimplikar SW, Nixon RA, Robakis NK, Shen J, Tsai LH. Amyloid-independent mechanisms in Alzheimer's disease pathogenesis. J Neurosci 2010;30:14946–54.

[7] Herrup K. Reimagining Alzheimer's disease–an age-based hypothesis. J Neurosci 2010;30:16755–62.

[8] Mawuenyega KG, Sigurdson W, Ovod V, Munsell L, Kasten T, Morris JC, et al. Decreased Clearance of CNS {beta}-Amyloid in Alzheimer's Disease. Science 2010;330:1774.

[9] Selkoe DJ. Alzheimer's disease is a synaptic failure. Science 2002; 298:789–91.

[10] Lee HG, Casadesus G, Zhu X, Takeda A, Perry G, Smith MA. Challenging the amyloid cascade hypothesis: senile plaques and amyloid-beta as protective adaptations to Alzheimer disease. Ann N Y Acad Sci 2004;1019:1–4.

[11] Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, et al. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med 2008; 14:837–42.

[12] Yaffe K, Fiocco AJ, Lindquist K, Vittinghoff E, Simonsick EM, Newman AB, et al. Predictors of maintaining cognitive function in older adults: the Health ABC study. Neurology 2009;72:2029–35.

[13] Arvanitakis Z, Wilson RS, Bienias JL, Evans DA, Bennett DA. Diabetes mellitus and risk of Alzheimer disease and decline in cognitive function. Arch Neurol 2004;61:661–6.

[14] Craft S. The role of metabolic disorders in Alzheimer disease and vascular dementia: two roads converged. Arch Neurol 2009;66:300–5.

[15] Ganguli M, Du Y, Dodge HH, Ratcliff GG, Chang CC. Depressive symptoms and cognitive decline in late life: a prospective epidemiological study. Arch Gen Psychiatry 2006;63:153–60.

[16] Wilson RS, Arnold SE, Schneider JA, Kelly JF, Tang Y, Bennett DA. Chronic psychological distress and risk of Alzheimer's disease in old age. Neuroepidemiology 2006;27:143–53.

[17] Onyike CU, Sheppard JM, Tschanz JT, Norton MC, Green RC, Steinberg M, et al. Epidemiology of apathy in older adults: the Cache County Study. Am J Geriatr Psychiatry 2007;15:365–75.

[18] Wilson RS, Scherr PA, Schneider JA, Tang Y, Bennett DA. Relation of cognitive activity to risk of developing Alzheimer disease. Neurology 2007;69:1911–20.

[19] Stern Y. Cognitive reserve. Neuropsychologia 2009;47:2015–28.

[20] Fotenos AF, Mintun MA, Snyder AZ, Morris JC, Buckner RL. Brain volume decline in aging: evidence for a relation between socioeconomic status, preclinical Alzheimer disease, and reserve. Arch Neurol 2008;65:113–20.

[21] Wilson RS, Barnes LL, Aggarwal NT, Boyle PA, Hebert LE, Mendes de Leon CF, et al. Cognitive activity and the cognitive morbidity of Alzheimer disease. Neurology 2010;75:990–6.

[22] Jack CR Jr, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol 2010;9:119–28.

[23] Reiman EM, Chen K, Alexander GE, Caselli RJ, Bandy D, Osborne D, et al. Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's dementia. Proc Natl Acad Sci U S A 2004; 101:284–9.

[24] Filippini N, MacIntosh BJ, Hough MG, Goodwin GM, Frisoni GB, Smith SM, et al. Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. Proc Natl Acad Sci U S A 2003;106:7209–14.

[25] Sheline YI, Morris JC, Snyder AZ, Price JL, Yan Z, D'Angelo G, et al. APOE4 allele disrupts resting state fMRI connectivity in the absence of amyloid plaques or decreased CSF Abeta42. J Neurosci 2010; 30:17035–40.

[26] Vemuri P, Wiste HJ, Weigand SD, Knopman DS, Trojanowski JQ, Shaw LM, et al. Serial MRI and CSF biomarkers in normal aging, MCI, and AD. Neurology 2010;75:143–51.

[27] Rowe CC, Ellis KA, Rimajova M, Bourgeat P, Pike KE, Jones G, et al. Amyloid imaging results from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging. Neurobiol Aging 2010; 31:1275–83.

[28] Mintun MA, Larossa GN, Sheline YI, Dence CS, Lee SY, Mach RH, et al. [11C]PIB in a nondemented population: potential antecedent marker of Alzheimer disease. Neurology 2006;67:446–52.

[29] Jack CR Jr, Lowe VJ, Senjem ML, Weigand SD, Kemp BJ, Shiung MM, et al. 11C PiB and structural MRI provide complementary information in imaging of Alzheimer's disease and amnestic mild cognitive impairment. Brain 2008;131(Pt 3):665–80.

[30] Gomperts SN, Rentz DM, Moran E, Becker JA, Locascio JJ, Klunk WE, et al. Imaging amyloid deposition in Lewy body diseases. Neurology 2008;71:903–10.

[31] De Meyer G, Shapiro F, Vanderstichele H, Vanmechelen E, Engelborghs S, De Deyn PP, et al. Diagnosis-independent Alzheimer

disease biomarker signature in cognitively normal elderly people. Arch Neurol 2010;67:949–56.
[32] Montine TJ, Peskind ER, Quinn JF, Wilson AM, Montine KS, Galasko D. Increased cerebrospinal fluid F(2)-isoprostanes are associated with aging and latent Alzheimer's disease as identified by biomarkers. Neuromolecular Med 2011;13:37–43.
[33] Arriagada PV, Marzloff K, Hyman BT. Distribution of Alzheimer-type pathologic changes in nondemented elderly individuals matches the pattern in Alzheimer's disease. Neurology 1992;42:1681–8.
[34] Morris JC, Storandt M, McKeel DW Jr, Rubin EH, Price JL, Grant EA, et al. Cerebral amyloid deposition and diffuse plaques in "normal" aging: evidence for presymptomatic and very mild Alzheimer's disease. Neurology 1996;46:707–19.
[35] Bennett D, Schneider J, Arvanitakis Z, Kelly J, Aggarwal N, Shah R, et al. Neuropathology of older persons without cognitive impairment from two community-based studies. Neurology 2006;66:1837–44.
[36] Brookmeyer R, Gray S, Kawas C. Projections of Alzheimer's disease in the United States and the public health impact of delaying disease onset. Am J Public Health 1998;88:1337–42.
[37] Alzheimer's Association. 2009 Alzheimer's disease facts and figures. Alzheimers Dement 2009;5:234–70.
[38] Moonis M, Swearer JM, Dayaw MP, St George-Hyslop P, Rogaeva E, Kawarai T, et al. Familial Alzheimer disease: decreases in CSF Abeta42 levels precede cognitive decline. Neurology 2005;65:323–5.
[39] Klunk WE, Price JC, Mathis CA, Tsopelas ND, Lopresti BJ, Ziolko SK, et al. Amyloid deposition begins in the striatum of presenilin-1 mutation carriers from two unrelated pedigrees. J Neurosci 2007;27:6174–84.
[40] Ringman JM, Younkin SG, Pratico D, Seltzer W, Cole GM, Geschwind DH, et al. Biochemical markers in persons with preclinical familial Alzheimer disease. Neurology 2008;71:85–92.
[41] Reiman EM, Chen K, Liu X, Bandy D, Yu M, Lee W, et al. Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. Proc Natl Acad Sci U S A 2009; 106:6820–5.
[42] Sperling RA, Laviolette PS, O'Keefe K, O'Brien J, Rentz DM, Pihlajamaki M, et al. Amyloid deposition is associated with impaired default network function in older persons without dementia. Neuron 2009;63:178–88.
[43] Hedden T, Van Dijk KR, Becker JA, Mehta A, Sperling RA, Johnson KA, et al. Disruption of functional connectivity in clinically normal older adults harboring amyloid burden. J Neurosci 2009; 29:12686–94.
[44] Sheline YI, Raichle ME, Snyder AZ, Morris JC, Head D, Wang S, et al. Amyloid plaques disrupt resting state default mode network connectivity in cognitively normal elderly. Biol Psychiatry 2010;67:584–7.
[45] Fjell AM, Walhovd KB, Fennema-Notestine C, McEvoy LK, Hagler DJ, Holland D, et al. Brain atrophy in healthy aging is related to CSF levels of Abeta1-42. Cereb Cortex 2010;20:2069–79.
[46] Dickerson BC, Bakkour A, Salat DH, Feczko E, Pacheco J, Greve DN, et al. The cortical signature of Alzheimer's disease: regionally specific cortical thinning relates to symptom severity in very mild to mild AD dementia and is detectable in asymptomatic amyloid-positive individuals. Cereb Cortex 2009;19:497–510.
[47] Desikan RS, Sabuncu MR, Schmansky NJ, Reuter M, Cabral HJ, Hess CP, et al. Selective disruption of the cerebral neocortex in Alzheimer's disease. PLoS One 2010;5:e12853.
[48] Becker JA, Rentz D, Carmasin JS, Hedden T, Hamdi I, Buckner RL, et al. Amyloid deposition and brain volume across the continuum of aging and AD. Ann Neurol 2011 (in press).
[49] Oh H, Mormino EC, Madison C, Hayenga A, Smiljic A, Jagust WJ. beta-Amyloid affects frontal and posterior brain networks in normal aging. Neuroimage 2011;54:1887–95.
[50] Pike KE, Savage G, Villemagne VL, Ng S, Moss SA, Maruff P, et al. Beta-amyloid imaging and memory in non-demented individuals: evidence for preclinical Alzheimer's disease. Brain 2007;130(Pt 11):2837–44.
[51] Mormino EC, Kluth JT, Madison CM, Rabinovici GD, Baker SL, Miller BL, et al. Episodic memory loss is related to hippocampal-mediated {beta}-amyloid deposition in elderly subjects. Brain 2009; 132:1310–23.
[52] Aizenstein HJ, Nebes RD, Saxton JA, Price JC, Mathis CA, Tsopelas ND, et al. Frequent amyloid deposition without significant cognitive impairment among the elderly. Arch Neurol 2008;65:1509–17.
[53] Rentz DM, Locascio JJ, Becker JA, Moran EK, Eng E, Buckner RL, et al. Cognition, reserve, and amyloid deposition in normal aging. Ann Neurol 2010;67:353–64.
[54] Villemagne VL, Pike KE, Chetelat G, Ellis KA, Mulligan RS, Bourgeat P, et al. Longitudinal assessment of Abeta and cognition in aging and Alzheimer disease. Ann Neurol 2011;69:181–92.
[55] Schott JM, Bartlett JW, Fox NC, Barnes J; Investigators ftAsDNI. Increased brain atrophy rates in cognitively normal older adults with low cerebrospinal fluid Aβ1-42. Ann Neurol 2011 (in press).
[56] Fagan AM, Roe CM, Xiong C, Mintun MA, Morris JC, Holtzman DM. Cerebrospinal fluid tau/beta-amyloid(42) ratio as a prediction of cognitive decline in nondemented older adults. Arch Neurol 2007;64:343–9.
[57] Li G, Sokal I, Quinn JF, Leverenz JB, Brodey M, Schellenberg GD, et al. CSF tau/Abeta42 ratio for increased risk of mild cognitive impairment: a follow-up study. Neurology 2007;69:631–9.
[58] Storandt M, Mintun MA, Head D, Morris JC. Cognitive decline and brain volume loss as signatures of cerebral amyloid-beta peptide deposition identified with Pittsburgh compound B: cognitive decline associated with Abeta deposition. Arch Neurol 2009;66:1476–81.
[59] Resnick SM, Sojkova J, Zhou Y, An Y, Ye W, Holt DP, et al. Longitudinal cognitive decline is associated with fibrillar amyloid-beta measured by [11C]PiB. Neurology 2010;74:807–15.
[60] Morris JC, Roe CM, Grant EA, Head D, Storandt M, Goate AM, et al. Pittsburgh Compound B imaging and prediction of progression from cognitive normality to symptomatic Alzheimer disease. Arch Neurol 2009;66:1469–75.
[61] Villemagne VL, Pike KE, Darby D, Maruff P, Savage G, Ng S, et al. Abeta deposits in older non-demented individuals with cognitive decline are indicative of preclinical Alzheimer's disease. Neuropsychologia 2008;46:1688–97.
[62] Chételat G, Villemagne VL, Pike KE, Ellis KA, Bourgeat P, Jones G, et al. Independent contribution of temporal b-amyloid deposition to memory decline in non-dementedelderly. Brain 2011;134:798–807.
[63] Vemuri P, Wiste HJ, Weigand SD, Shaw LM, Trojanowski JQ, Weiner MW, et al. MRI and CSF biomarkers in normal, MCI, and AD subjects: predicting future clinical change. Neurology 2009;73:294–301.
[64] Yaffe K, Weston A, Graff-Radford NR, Satterfield S, Simonsick EM, Younkin SG, et al. Association of plasma beta-amyloid level and cognitive reserve with subsequent cognitive decline. JAMA 2011;305:261–6.
[65] Howieson DB, Carlson NE, Moore MM, Wasserman D, Abendroth CD, Payne-Murphy J, et al. Trajectory of mild cognitive impairment onset. J Int Neuropsychol Soc 2008;14:192–8.
[66] Shaw LM, Vanderstichele H, Knapik-Czajka M, Clark CM, Aisen PS, Petersen RC, et al. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. Ann Neurol 2009; 65:403–13.
[67] Fagan AM, Mintun MA, Mach RH, Lee SY, Dence CS, Shah AR, et al. Inverse relation between in vivo amyloid imaging load and cerebrospinal fluid Abeta42 in humans. Ann Neurol 2006;59:512–9.
[68] Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. Acta Neuropathol 1991;82:239–59.
[69] Hebert LE, Scherr PA, Beckett LA, Albert MS, Pilgrim DM, Chown MJ, et al. Age-specific incidence of Alzheimer's disease in a community population. JAMA 1995;273:1354–9.
[70] Ganguli M, Dodge HH, Chen P, Belle S, DeKosky ST. Ten-year incidence of dementia in a rural elderly US community population: the MoVIES Project. Neurology 2000;54:1109–16.
[71] Kukull WA, Higdon R, Bowen JD, McCormick WC, Teri L, Schellenberg GD, et al. Dementia and Alzheimer disease incidence: a prospective cohort study. Arch Neurol 2002;59:1737–46.

アルツハイマー病治療の今後の展望
－予防介入の可能性

Perspectives on the future therapeutics of Alzheimer disease

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Summary

アルツハイマー病(AD)の基礎研究の発展に伴い，メカニズムに基づく疾患修飾薬の開発が進行中である。また，ADNIなどの画像・バイオマーカー研究により，その効果の客観的評価指標が確立しつつある。このような動きを背景に，AD治療は早期に向かって展開し，軽度認知障害期のprodromal ADに対する治療，そして認知機能の正常なpreclinical ADに対する予防・治療が視野に入りつつある。

Key words

●アルツハイマー病
●disease-modifying therapy
●予防
●prodromal AD
●preclinical AD
●バイオマーカー

Ⅰ はじめに

アルツハイマー病(Alzheimer's disease；AD)の治療法は，症候改善療法(symptomatic therapy)と疾患修飾療法(disease-modifying therapy；DMT)に大別される。症候改善療法としてはすでにアセチルコリンエステラーゼ阻害薬とメマンチンが臨床応用されてきたが，2011年，ガランタミンその他の薬剤が本邦でも認可され，海外と同じ4剤のラインアップが揃う見込みであることは喜ばしい。

一方，ADの病態メカニズムに即して，神経変性すなわち疾患そのものの進行を遅らせようとするのがDMTである。過去四半世紀の間にADの病態メカニズム研究は長足の進歩を遂げ，アミロイドβペプチド(Aβ)のAD発症，タウ蛋白質の神経細胞死における原因的役割がそれぞれ実証された。ことにAβについては，産生プロテアーゼの阻害や，Aβに対する免疫療法など，すでに臨床レベルでのさまざまな薬剤開発が進行中である。本稿ではdisease-modificationを目指したAD治療の将来展望，ことに将来の主要な方向性と目される予防介入の成功に向けてのロードマップと，課題について論じたい。

Ⅱ アルツハイマー病の病態と疾患修飾療法の標的分子

ADの病理学的特徴である老人斑アミロイドを構成するAβは，前駆体蛋白質APP(amyloid precursor protein)

から，βセクレターゼ(beta-site APP-cleaving enzyme 1；BACE1)，γセクレターゼによる連続した切断を受けて生成される。Aβは生理的にはネプリライシン，IDE (insulin degrading enzyme)などの活性により分解され消失するが，ADでは正常な代謝を逸脱し，細胞外腔にアミロイド線維として蓄積する。常染色体優性遺伝を示す家族性AD(FAD)の病因遺伝子としてAPP，ならびにγセクレターゼの酵素本体をコードするプレセニリンが同定され，それらの変異は，いずれもAβの凝集・蓄積を促進することが示された。またADの強力な危険遺伝子となるアポリポ蛋白E(apoE)のε4アレルも，Aβ凝集促進作用をその病的効果の1つとして有することが，*in vivo*の実験により示された。これらの知見から，Aβの蓄積過程はADに対して病因的に働くこと(正確な意味での「アミロイド仮説」)が立証されたのである。また，タウ蛋白質の神経原線維変化としての細胞内蓄積は，AD脳における神経細胞脱落とよく相関することが知られてきたが，タウ蓄積を伴う家族性神経変性疾患FTDP-17(frontotemporal dementia and parkinsonism linked to chromosome 17)が，タウ遺伝子変異により生じることが示され，タウ蛋白質の蓄積は，ADにおいても神経細胞死に原因的に働くことが強く示唆された。これらの知見から，Aβとタウという二大神経病理学的特徴は，ADの治療ターゲットとも目されるに至った。さらにAD脳は，ミクログリアの活性化を伴う特殊な慢性炎症状態にあること，酸化ストレスレベルが高いことも知られ，グルタミン酸による興奮毒性などとともに，炎症，酸化ストレスも治療標的と想定されている。

III　疾患修飾療法のストラテジーとアルツハイマー病における疾患修飾療法の臨床治験

種々のDMTのうち，最も早期から開発が進んだのが，抗Aβ療法である。ことに，Aβの産生に関わるβ，γセクレターゼの阻害薬のうち，γセクレターゼ阻害薬は早くから開発が進んだ。γセクレターゼの生理的基質として発生・分化に関わるNotchが同定されたことから，Notch切断を温存する"Notch sparing inhibitor"[1]，またAβ42の産生を特異的に阻害する「γセクレターゼモジュレータ」も開発されている。βセクレターゼ阻害薬は，BACE1の触媒ポケットの構造が浅く広いことから開発が遅れたが，最近になり低分子性阻害薬の創出が進んでいる[2]。またAβペプチドの能動ワクチン療法に端を発する「Aβ免疫療法」が開発され，現在抗Aβ抗体の受動免疫を中心に複数の臨床治験が行われている。このほかAβの凝集阻害薬も開発されているが，tramiprosate (Alzhemed®)は2008年第3相治験の不成功が報じられた。

この中で最も大規模な臨床治験が進められたのが，イーライリリー社のγセクレターゼ阻害薬semagacestatである。しかし2010年8月，実薬群において偽薬群に比して，認知機能障害の進行が有意に高かったことを主な理由に，第3相試験が突如中止されるに至った[3]。詳細なデータはまだ公表されておらず，その原因は2011年中に詳細に解析される見込みであるが，Notch阻害による種々の副作用などの関与が議論されている。また，抗Aβ抗体bapineuzumabの第2相試験結果が2008年に公表されているが，サンプル数の少なさもあり，認知機能の改善は全般には有意でなく，用量依存性も明確に示されなかった[4]。

これらの結果は，認知機能改善のみを評価指標とする従来型の臨床治験を，DMTに適用することの困難さを物語っている。従来から，DMTが18ヵ月程度の一般的な治験期間のうちに臨床症状改善効果を示すかどうかには疑問ももたれてきた。また，現在のAD治療薬臨床治験は，認知症の症状が完成した，「臨床的AD」の発症後に行うことが標準的であり，従来は規制当局もそれを要請してきた。しかしながら，ADの病理学的変化が，認知症症状の初発にはるかに先行して出現すること，そしてDMTの多くはこれらの病理学的変化の進行を標的とすることからみて，DMTのより好適な対象は，神経細胞変性が相当度に進行した「臨床的AD」以前の時期にあるのではないかとの見方も強まっている。

次節で述べるように，ADの進行過程において，dementiaの出現以前に「軽度認知障害」(mild cognitive impairment；MCI)の臨床期を経ることが認識されるよう

になった。すなわち，ADのDMTは，MCIからADへの進展(conversion)の予防に向けられるべき可能性も高い。これを実現するためには，初期臨床症状の鋭敏な検査法とともに，画像，体液生化学などを駆使したバイオマーカーを制定し，進行予測，進行度評価などに役立てることが必要となる。この目的で，MCIからADへの進行過程を画像・バイオマーカーで計量的に評価する観察研究Alzheimer's Disease Neuroimaging Initiative (ADNI)が米国ならびに本邦で進められている[5]。

Ⅳ MCI due to AD(prodromal AD)に対する疾患修飾療法

MCIは，健忘などの認知機能障害が存在するが，dementiaに至っていない，「正常と認知症の中間状態」に相当する。MCIの背景病理は均一ではないが，記憶障害が前景に立ち，進行性の強い"amnestic MCI"例はADを背景病理とし，臨床的にも早期に認知症を発症する頻度が高い。ことに近年，アミロイドPETや脳脊髄液Aβ(1-42)，タウ，MRIでの内側側頭葉萎縮など，ADの病理を反映する変化を画像・バイオマーカーにより予測することが可能となった。このため，ADの病態概念を，dementiaの完成した従来のADよりも早期へと拡張する動きが急となっている。2011年，米国国立老化研究所(National Institute on Aging；NIA)とアルツハイマー協会は，合同で新たなADの研究用診断基準を制定する見込みである。これによれば，AD dementia，MCI due to AD(これはprodromal ADと呼ばれることもある)に加えて，臨床症状は皆無もしくはminimalであるが，アミロイド蓄積などのADの病理変化の推定される超初期状態として"preclinical AD"の3段階が区別される[6]。

これまでにamnestic MCIに対するアセチルコリンエステラーゼ阻害薬の臨床治験が行われているが，ドネペジル[7]，ガランタミン[8]ともに，部分的な症候改善効果は示したものの，疾患修飾効果(conversion率の改善)は認められていない。

DMTについても，MCI/prodromal ADに対する大規模臨床治験はまだ端緒についたばかりであり，ブリストルマイヤーズ社が，Notch温存性γセクレターゼ阻害薬の第2相試験に着手したと報じられるのみである。MCI期には認知機能低下，MRIによる脳萎縮などの画像変化の進行速度は，いずれもAD発症後よりも緩徐であるため，認知機能のみをエンドポイントとする評価は困難となる。米国ADNI1の結果に基づいて，髄液・アミロイドPETなどによりconverter候補(すなわちAD病理を有する被験者)を絞り込み，CDR(Clinical Dementia Rating) sum of boxなどによる認知機能評価に，MRI脳容積変化を共変数として組み合わせることにより，仮に年間40％の効果を有する薬剤があれば，実薬群・偽薬群各アーム100～200例のサイズでMCI/prodromal ADの治験を組み立てることが可能と試算されている[9]。

このようにMCIの一部を占める，ADに進展する例を"prodromal AD"と位置づけ，治験レベルで薬剤介入の対象となる疾病と位置づけることの医学的な妥当性は，欧米を中心にほぼ確立しつつある。本邦においても，今後prodromal ADに対する臨床治験を推進するために，J-ADNIなどの系統的な臨床研究により収集されたamnestic MCI例を分析し，その位置づけ，海外症例との等価性を確定することが急務であろう。

Ⅴ 前臨床期アルツハイマー病に対する予防的介入の展望

従来，疫学的研究から，ADのリスク低減因子として，非ステロイド抗炎症薬，スタチン，食事，運動などが指摘されてきた。しかし，これらの因子の制御による予防的治験が完遂された例はほぼ皆無である[10]。その理由としては，認知機能障害発症などの臨床指標を用いた予防介入試験は莫大な被験者数と観察期間を要し，試験デザインにも課題の多いこと，またAD発症リスクの高い被験者の選択が困難であったことが挙げられよう。

ADの病理学的変化は，MCI/prodromal ADの時期よりもさらに先行して生じると考えられる。その最初期変化の1つであるAβ蓄積は，今日アミロイドPETや脳脊髄液Aβ(1-42)低下により検出可能である。Aβ とAD発症の因果関係が最も明確な，優性遺伝性FAD家

系の未発症保因者を含む多数のメンバーを登録，検討する米国の臨床研究“Dominantly Inherited Alzheimer Network”（DIAN）における発症前期の観察からは，アミロイド蓄積のみの時期が約10年間，minimalな認知機能障害が進行してdementiaに至るまでにさらに10年間が経過するものと予測されている（Morrisら，第10回AD/PD カンファレンス 2011）。一般高齢者を対象とした最近のアミロイドPET臨床研究の結果では，認知機能障害のない高齢者の20～30％程度にアミロイドが検出されており，J-ADNIでも同様の結果が得られつつある[11][12]。もっとも，これらの無症候アミロイド陽性者のすべてが，その生涯のうちにMCI，ADに進展するとは考えにくい。アミロイド陽性を示した個人が，十数年続くと考えられるアミロイド陽性無症候期の中でいかなるステージに位置し，その生涯のうちに認知機能障害を発症する危険がどの程度かを判定することが，今後の超早期予防介入には必須であろう。そのためには，多数のアミロイド陽性無症候者の長期間にわたる縦断的観察研究が必須であるが，まだ十分な継続期間と観察数を満たした報告はほとんどない。2009年ワシントン大セントルイスのMorrisらは，159例の認知機能正常（CDR 0）高齢者にアミロイドPETを施行し，0.8～5.5年の観察期間中にCDR 0.5に進展した23例（うち9例は臨床的にADと診断）を解析，アミロイド陽性者では陰性者に比し，進行リスクが4.8倍増大したこと，進行群では海馬傍回の萎縮がより強かったことを報告している[13]。Presymptomatic FADの縦断的な観察結果を含む諸知見に基づき，NIA/アルツハイマー協会の研究用新診断基準では，アミロイド蓄積のみが見出される無症候期を，preclinical ADと定義している[6]。Preclinical ADはさらに，①純粋にアミロイド蓄積のみ，②脳萎縮・タウ上昇など細胞変性徴候があるが無症候，③minimalな認知機能障害発症，の3段階を経ると仮想されている。

2010年に入り，preclinical ADに対する薬剤介入研究（病理陽性者に対する介入としては二次予防と捉えることもできる）が本格的に検討されるようになった。カリフォルニア大学サンディエゴ校に設置されている全米AD医師主導治験のセンターAlzheimer's Disease Cooperative Study（ADCS）のAisenらは，アミロイドPETもしくは脳脊髄液検査からアミロイド陽性が示唆される認知機能健常高齢者に対し，抗アミロイド薬を投与する薬剤介入臨床研究Anti-Amyloid treatment in Asymptomatic AD（A4）をNIH（National Institute of Health）に申請することを計画中である。この場合，認知機能に代わる評価指標として，MRIで計測した海馬などの脳容積の変化を用いることが想定されている。その背景には，米国ADNI1において，認知機能健常高齢者のMRI volumetryにより，髄液Aβ（1-42）低値を示す被験者の年間海馬萎縮率は3.6％と，正常者の2.2％に比して60％の加速が実証されたことがある[14]。この数値に基づけば，海馬萎縮率の改善を指標に，抗Aβ薬の脳萎縮進行に対する効果判定が可能となる。年間25％の萎縮改善効果を有する薬剤の効果は，1アーム316例のアミロイド陽性健常者を検討することにより統計学的な検証が可能とされる。

これに対しアリゾナ州Banner研究所のReimanらは，ADの危険遺伝子であるapoEε4アレルのホモ接合体保有者（ADの発症リスクがε3ホモ接合体に比して十数倍増大する），および南米コロンビアのプレセニリン1 E280A変異大家系の未発症者に，薬剤介入を行うAlzheimer's Disease Prevention Initiative（API）を推進している[15]。ワシントン大セントルイスのDIANも，家族性ADの未発症キャリアに抗Aβ薬を投与する介入臨床試験を計画中である[16]。筆者らも，J-ADNIなどを基盤に，アミロイドPET，脳MRI容積測定を用いた抗Aβ薬による介入・先制医療研究について，準備を進めている。

Ⅵ　おわりに

AD発症機構の研究に基づく，mechanism-basedなDMTの開発は，dementiaを発症したADでは難航しているが，画像・バイオマーカーを用いた超早期ADに関する種々の臨床研究の結果から，prodromal ADさらにはアミロイド陽性無症候者（preclinical AD）における予防介入まで，そのコンセプトは急速に広がりつつある。このような「超早期治療」を近未来に行う場合，従来の

概念では健常者に属する被験者に，安全性と効能が完全に証明されていない新薬を長期投与する，という障壁が生じる。これを可能とするためには，長期投与における安全性と，メカニズムに基づく強い薬効が保証された優れた薬剤を，製薬企業とアカデミック，そして社会が一体となって育てる体制が必要となる。また，これらのDMTが真のAD治療・予防薬となるためには，画像を含むバイオマーカーの改善のみではなく，最終的に臨床的な改善がもたらされることが条件となることはいうまでもない。

抗Aβ薬のみならず，抗タウ薬，抗炎症薬・抗酸化薬などあらゆるDMTは，超早期において最大の効果を有する可能性がある。コレステロール降下薬や降圧薬を健常時から投与し，動脈硬化や血管イベントを予防することが現代の日常臨床で行われているように，アミロイドをはじめとするADの危険因子を薬剤介入によりコントロールすることにより，ADの予防が実現される日の遠くないことを切望するものである。

文　献

1）Tomita T, Wong PC：Selectivity to amyloid-β precursor protein cleavage provides hope against Alzheimer's. Alzheimers Res Ther **3**：7, 2011
2）Fukumoto H, Takahashi H, Tarui N, et al：A non-competitive BACE1 inhibitor TAK-070 ameliorates Aβ pathology and behavioral deficits in a mouse model of Alzheimer's disease. J Neurosci **30**：11157-11166, 2010
3）Schor NF：What the halted phase Ⅲ γ-secretase inhibitor trial may (or may not) be telling us. Ann Neurol **69**：237-239, 2011
4）Salloway S, Sperling R, Gilman S, et al：Bapineuzumab 201 Clinical Trial Investigators；A phase 2 multiple ascending dose trial of bapineuzumab in mild to moderate Alzheimer disease. Neurology **73**：2061-2070, 2009
5）Iwatsubo T：Japanese ADNI；Present status and future. Alzheimers Dement **6**：297-299, 2010
6）Sperling RA, Aisen PS, Beckett LA, et al：Toward defining the preclinical stages of Alzheimer's disease；Recommendations from the National Institute on Aging and the Alzheimer Association Workgroup. Alzheimers Dement 2011 (in press)
7）Petersen RC, Thomas RG, Grundman M, et al：Alzheimer's Disease Cooperative Study Group. Vitamin E and donepezil for the treatment of mild cognitive impairment. N Engl J Med **352**：2379-2388, 2005
8）Winblad B, Gauthier S, Scinto L, et al：GAL-INT-11/18 Study Group：Safety and efficacy of galantamine in subjects with mild cognitive impairment. Neurology **70**：2024-2035, 2008
9）Weiner MW, et al [http：//www.adni-info.org/Scientists/Pdfs/Weiner_ NINDS_20101020.pdf]
10）Andrieu S, Coley N, Aisen P, et al：Methodological issues in primary prevention trials for neurodegenerative dementia. J Alzheimers Dis **16**：235-270, 2009
11）Jack CR Jr, Lowe VJ, Senjem ML, et al：11C PiB and structural MRI provide complementary information in imaging of Alzheimer's disease and amnestic mild cognitive impairment. Brain **131**：665-680, 2008
12）Morris JC, Roe CM, Xiong C, et al：APOE predicts amyloid-β but not tau Alzheimer pathology in cognitively normal aging. Ann Neurol **67**：122-131, 2010
13）Morris JC, Roe CM, Grant EA, et al：Pittsburgh compound B imaging and prediction of progression from cognitive normality to symptomatic Alzheimer disease. Arch Neurol **66**：1469-1475, 2009
14）Aisen P：Treating before symptoms－ADCS invites ideas for clinical trials in very early AD. Web discussions at Alzforum. June 9, 2010 [http：//www.alzforum.org/res/for/journal/detail.asp?liveID=180]
15）Strobel G：Time to open the kimono－Which drugs in preclinical trials？[http：//www.alzforum.org/new/detail.asp?id=2730]
16）Bateman RJ, Aisen PS, De Strooper B, et al：Autosomal-dominant Alzheimer's disease；A review and proposal for the prevention of Alzheimer's disease. Alzheimers Res Ther **3**：1, 2011

特報　第48回 2011年度 ベルツ賞受賞論文 2等賞

アルツハイマー病
—βアミロイドをめぐる分子病態と先制医療への展望—

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Summary

Alzheimer's disease (AD) is a devastating dementing neurodegenerative disorder affecting millions of elderly people in Japan and worldwide, and there is a compelling need for new therapies that retard the progression of AD. Neuropathology of AD is characterized by loss of cerebrocortical neurons associated with accumulation of tau-rich neurofibrillary changes and amyloid (senile) plaques. Deposition of amyloid β peptides (Aβ) as senile plaques is the most characteristic feature of AD, which is implicated in its pathogenesis and deemed as the prime target for the mechanism-based, disease-modifying therapy (DMT). Aβ deposition is determined by the production and clearance. Aβ is produced by sequential proteolytic cleavages by two membrane-associated proteases termed β- and γ-secretases. γ-Secretase, harboring presenilins (PS) as the catalytic center subunit, forms the C terminus of Aβ, the latter being the determinant for its propensity to aggregate: missense mutations in PS genes cause familial AD by altering the preferred γ-secretase cleavage sites in a way to increase production of pathogenic Aβ42 species. γ-Secretase is composed of four membrane proteins, i.e. PS, nicastrin, APH-1 and PEN-2, and its complex composition and structure buried within the membrane hampered detailed analysis of the mechanisms whereby hydrolysis of transmembrane substrates is executed within the lipid bilayer. We found that γ-secretase forms a hydrophilic pore within the membrane, which enables the unique mode of intramambrane proteolysis to form Aβ. We showed that a γ-secretase modifier GSM-1 interacts with the transmembrane domain 1 of PS1 to selectively lower the production of Aβ42. We also found that the transmembrane domain of BACE1, the *bona fide* β-secretase, is the target structure of sphingosine-1-phosphate that enhances the BACE1 activity, as well as of a novel BACE1 inhibitor, TAK-070. Another Aβ-lowering therapy, i.e., Aβ immunotherapy, facilitates the clearance of Aβ from brain parenchyma through the activities of anti-Aβ antibodies that entered the brain parenchyma, contrary to the assumption of a "sink effect" to pull out Aβ into the blood. To clinically develop the

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DMTs for AD, establishment of imaging and fluid biomarkers that surrogate the AD pathology is mandatory, which is underway through the ADNI clinical studies. Collectively, these basic and clinical approaches to the disease-modification of AD will surely pave the way towards the pre-emptive medicine for AD, enabling retardation of the progression of neurodegeneration and preventing the clinical manifestations of dementia.

緒　論

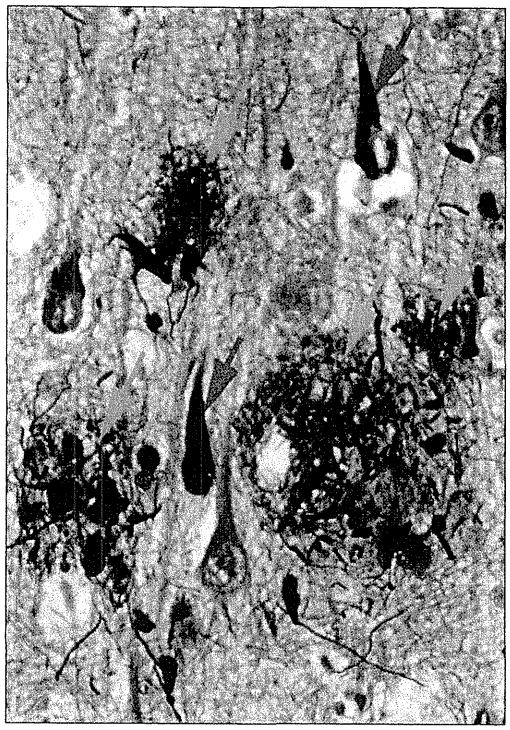
アルツハイマー病（Alzheimer's disease：以下 AD と略す）は，老年期認知症の原因として最も頻度の高い神経変性疾患である．現在本邦の認知症患者数は 65 歳以上の高齢者の 10% を超えるとの報告もなされ，その 2/3 前後が AD に起因するものとすれば，総患者数は 200 万人を超える．高齢化の進行とともに AD の発症は増加しており，根本的治療法の開発なくしては，2050 年には本邦でも患者数は 500 万人を超えると予想されている．現在，世界の AD による経済損失額は邦貨換算で 50 兆円を超え，本邦でも介護者の負担などを含む社会経済損失は 5 兆円に達するといわれ，介護保険制度をはじめとする医療を圧迫し始めている．AD の発症機序の解明と，それに基づく根本的治療法（disease-modifying therapy：疾患修飾療法）の実用化は，まさに焦眉の急である．

AD は 1906 年，ベルツの母校でもあるチュービンゲン大学における Alois Alzheimer による臨床病理学的報告において，初めて記載された[1]．51 歳で入院，56 歳で死亡したこの AD の初例は，現代では「初老期認知症（presenile dementia）」に分類される症例であり，高齢化社会とともに急増しつつある老年期発症例は "senile dementia of Alzheimer type"（SDAT）と呼ばれることもあった．しかし 1970 年代以降，米国の Katzman らの提唱により，初老期認知症としての AD と，老年期に発症する SDAT の間に多くの臨床病理学的共通点があることを重視する見方が定着し，現在では両者を一括して AD として扱うことが多い．

AD は，病理学的に大脳皮質を中心とする神経細胞の脱落，神経細胞内の神経原線維変化の蓄積と，老人斑の形成を特徴とする（図 1）[2]．AD における認知機能障害は，基本的に，神経細胞死に伴う神経回路障害に起因するものと考えられる．2 種類の蓄積性病変のうち，神経原線維変化は微小管結合タンパク質であるタウが重合して生じ，神経細胞死に関連することが知られている．老人斑は，細胞外腔におけるアミロイド線維を骨格として，神経突起，グリア細胞の反応性変化を伴う斑状病変で，アミロイドは血管壁にも蓄積する（脳血管アミロイド）．老人斑アミロイドは Aβ ペプチドをその構成成分とし，AD に特異性が高いこと，AD の病理学的変化のうち最も早期に出現することから，AD の病因に関係が深い変化と考えられてきた．また次章で述べるように，家族性 AD の病因遺伝子が種々の形式で Aβ の凝集を亢進させる作用を有することから，Aβ 蓄積を AD の病因と考える「β アミロイド仮説」が広く支持されるに至った[3]．

本論文では，我々がこれまでに行ってきた，Aβ を標的とする AD の分子病態研究の成果に基づいて，病因メカニズムを標的とする疾患修飾療法，ことに臨床症状の発症前の「先制医療」（pre-emptive medicine）の実現に向けての課題を含めて論じたい．

図1　アルツハイマー病の病理所見
(Bielshowsky 鍍銀染色)

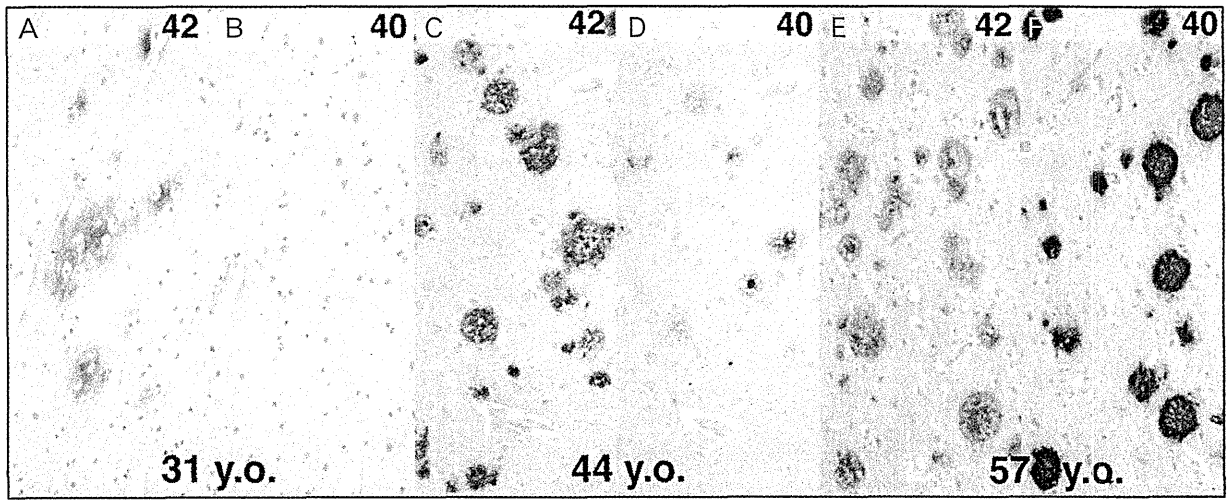


神経細胞脱落に加えて，神経原線維変化（ピンク色矢印），老人斑（緑色矢印）の出現を特徴とする．52歳女性例，海馬 CA1 領域．

アルツハイマー病における脳アミロイドの病理学・生化学的解析

1984年 Glenner らは，脳血管アミロイドの成分として分子量約4 kDa のペプチドを同定し，β ペプチド（後に Aβ）と命名した[4]．翌1985年 Beyreuther，Masters らは，老人斑アミロイドもまた Aβ からなること，そして Aβ のカルボキシ（C）末端には，40番のバリンで終止する Aβ40 と42番のアラニンまで伸びた Aβ42 があることを，アミノ酸配列解析により同定した[5]．1993年 Lansbury らは，Aβ42 は Aβ40 に比してアミロイド線維形成の「凝集核」を形成しやすく，その結果アミロイド線維の形成を促進するとの *in vitro* 実験から，C末端長のバリエーションに病理学的な意味があることを示唆した[6]．我々はヒト脳のアミロイド形成過程において，Aβ40 と Aβ42 の蓄積過程に遅速があるのかに興味を持った．しかしその検証には2つの障壁があった．第1に，認知症症状の完成した臨床的な AD の発症後は，病理学的には AD の進行期に相当し，症状発現直後と晩期の症例を比較しても病変の進行過程を知ることは困難である．この問題は，若年期にびまん性老人斑の形でアミロイド蓄積の初期像を発症し，加齢とともに老人斑の成熟，神経原線維変化を含む神経細胞病変を発症し，AD の病理過程を数十年の間に再現することが知られている Down 症脳を系統的に観察することにより克服された．第2の問題は，Aβ40，Aβ42 の近接したC末端を識別することの困難さであった．この点は，90年代に応用可能性が広く知られるようになった，ペプチド・タンパク質の「切断部位認識抗体」の利用により解決された．我々は武田薬品工業つくば研究所の鈴木伸宏博士，浅見麻乃博士との共同研究により，Aβ40 特異抗体 BA27，Aβ42 特異抗体 BC05 を樹立し，AD ならびに Down 症脳の免疫組織化学を施行した（図2）．若年期（30歳前後）の Down 症大脳皮質には多数のびまん性老人斑が出現するが，これらは Aβ42 陽性を示すものの，Aβ40 には全く陰性である．ところが40歳を過ぎた Down 症脳には，よりコンパクトでアミロイド芯を有する典型的老人斑が出現し始め，これらは Aβ42 陽性であるが，部分的に Aβ40 陽性を示し始める．そして50代以降の，神経細胞脱落，神経原線維変化を伴い，密度の高い大型の老人斑が多発する AD 病理の完成した時期には，多くの老人斑が Aβ42 とともに Aβ40 に強陽性を示す．このようにヒトの AD 病理過程においても，Aβ42 が先行して蓄積を開始し，遅れて Aβ40 が蓄積するという時系列が確認され，蓄積タンパク質の一次配列の微細な相違に基づく物性（凝集性）の差異がアミロイド蓄積過程に反映される，という興味深い現象が実証された[7][8]．我々は西道らとの共同研究により，Aβ のアミノ（N）末端にも着目し，老人斑として蓄積する Aβ のN末端にも多様性が存在し，特にN末端2アミノ酸が欠損しグルタミン酸3より始まる Aβ のN末端は，初

図2　Down 症脳における Aβ42, Aβ40 蓄積の経時変化



若年（31 歳例）では，びまん性老人斑は Aβ42 陽性（A）だが Aβ40 陰性（B）である．40 代に入り Aβ42 陽性斑（C）の一部が Aβ40 陽性を示し始め（D），50 代後半に至って Aβ42 陽性斑（E）の多くが Aβ40 陽性を示すようになる（F）．A/B, C/D, E/F はそれぞれ前頭葉新皮質の連続切片．Aβ42 は BC05, Aβ40 は BA27 により免疫組織化学を行った．

期から高度にピログルタミル化を受け，分解に抵抗性を獲得していることを示唆した[9)10)]．

家族性 AD 病因遺伝子変異の解析 —APP とプレセニリンの役割—

Aβ の病因的意義に関する決定的な証拠は，病理生化学的解析と並行して，家族性 AD の遺伝子解析からもたらされた．早期発症型 AD（緒論において述べた初老期認知症型の AD に同義）の一部には，常染色体性優性遺伝形式をとる家族性 AD（以下 FAD）家系が存在する．FAD の病因遺伝子として最初に同定されたのは，Aβ の前駆体タンパク質として同定されていたアミロイド前駆体タンパク質（APP）であった（図3左）．APP 遺伝子は 21 番染色体上に存在し，695〜770 アミノ酸からなるⅠ型1回膜貫通タンパク質 APP をコードする[11)]．APP のアミノ酸配列中で Aβ は，膜近傍の細胞外 28 アミノ酸とそれに引き続く膜中央部までの 40〜42 アミノ酸に相当する（図3左）．即ち，Aβ のN末端部分は細胞外，C末端部分は膜内で切断を受け，Aβ ペプチドが生成される．これらのプロセシングはそれぞれ β 切断，γ 切断と仮称され，β セクレターゼ，γ セクレターゼという本態未同定のタンパク質分解酵素活性が関与するものと推定された．

1991 年，Hardy らにより最初に発見された London 型変異は，APP の膜内部分で，Aβ のC末端（γ セクレターゼ切断部位）よりさらに数残基外側に位置するバリン 717（Aβ の 46 位）をイソロイシンに変化させものであった[12)]．翌 1992 年スウェーデンで発見された第2の変異は，Aβ のN末端（β セクレターゼ切断部位）に隣接する2アミノ酸（リジン 670 およびメチオニン 671）が，アスパラギン，ロイシンに二重置換するものであった[13)]．Selkoe ら[14)]，Younkin ら[15)]は，Sweden 型変異 APP では β セクレターゼによる切断効率が上昇し，Aβ の産生が5〜8倍に顕著に上昇することを示した．一方 London 型変異では，切断部位の近接した γ セクレターゼ活性は上昇を示すことはなく，当初その本態は謎に包まれていた．London 型変異のアミノ酸置換部位が γ セクレターゼ切断部位に近接していること，Aβ42 の凝集性が高いことから，γ セクレターゼによる Aβ C末端の切り分けに影響が及ぶ可能性が浮上した．鈴木，Younkin らは，Aβ40, 42 を区別して測定可能な高感度サンドウィッチ ELISA を用い