Neuroimaging Biomarkers for Clinical Trials of Disease-Modifying Therapies in Alzheimer's Disease

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Summary: The pathophysiologic process leading to neurodegeneration in Alzheimer's disease (AD) is thought to begin long before clinical symptoms develop. Existing therapeutics for AD improve symptoms, but increasing efforts are being directed toward the development of therapies to impede the pathologic progression of the disease. Although these medications must ultimately demonstrate efficacy in slowing clinical decline, there is a critical need for biomarkers that will indicate whether a candidate disease-modifying therapeutic agent is actually altering the underlying degenerative process. A number of *in vivo* neuroimaging techniques, which can reliably and noninvasively assess aspects of neuroanatomy, chemistry, physiology, and pathology, hold promise as biomarkers. These neuroimaging measures appear to relate closely to neuropathological and clinical data, such as rate of cognitive decline and risk of future decline. As this work has matured, it has become clear that neuroimaging measures may serve a variety of potential roles in clinical trials of candidate neurotherapeutic agents for AD, depending in part on the question of interest and phase of drug development. In this article, we review data related to the range of neuroimaging biomarkers of Alzheimer's disease and consider potential applications of these techniques to clinical trials, particularly with respect to the monitoring of disease progression in trials of disease-modifying therapies. **Key Words:** Alzheimer's disease, mild cognitive impairment, magnetic resonance imaging, positron emission tomography, single-photon emission tomography, clinical drug trials, biomarkers.

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia,[1] and it is the fourth leading cause of death in the United States. In the vast majority of individuals, symptoms begin after the sixth decade of life. The disease typically starts with mild memory difficulties and progresses insidiously, leading eventually to cognitive impairment that interferes with complex activities of daily life and ultimately results in the loss of independent function. Current treatments are primarily symptomatic, in that clinical trials demonstrate short-term improvement in cognitive function but not clearly a slowing of the rate of decline.[2] Increasing emphasis is being placed on the development of disease-modifying therapies—drugs that impede the underlying pathophysiologic process of neurodegeneration in AD and thereby slow the rate of cognitive decline. Extensive efforts are being directed toward the identification of candidate molecules in animal models of AD, and several clinical trials of putative disease-modifying therapies are now underway. At present, the potential efficacy of disease-modifying therapies is evaluated primarily using clinical measures of cognition and behavior. In animal models, traditional behavioral assessments are often used, such as the rate at which rodents learn to navigate a maze. In clinical trials, outcome measures are typically performance-based cognitive instruments, such as the Alzheimer's Disease Assessment Scale (ADAS-Cog),[3] or structured surveys of clinician/caregiver impression of change.[4]

Although the efficacy of disease-modifying treatments for AD must ultimately be demonstrated using clinically meaningful outcome measures such as the slowing of decline in cognitive function, such trials will likely require hundreds of patients studied for a minimum of 1–2 years. Thus, surrogate markers of efficacy with less variability than clinical assessments are desperately needed

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to reduce the number of subjects. These markers may also prove particularly valuable in the early phases of drug development to detect a preliminary "signal of efficacy" over a shorter time period.

Because the pathophysiologic process underlying cognitive decline in AD involves the progressive neurodegeneration of particular brain regions, repeatable *in vivo* neuroimaging measures of brain anatomy, chemistry, physiology, and pathology hold promise as an important class of potential biomarkers. A growing body of data indicates that the natural history of gradually progressive cognitive decline in AD can be reliably related to changes in such imaging measures. Furthermore, regionally specific changes in brain anatomy, chemistry, and physiology can be detected by imaging before the point at which the disease is symptomatic enough to make a typical clinical diagnosis. Finally, evidence is accumulating that alterations in synaptic function are present very early in the disease process, possibly long before the development of clinical symptoms and significant cell loss, which may relate closely to symptomatic progression in manifest disease.[5,6] Thus, potential disease-modifying therapies may act by impeding the accumulation of neuropathology, slowing the loss of neurons, altering neurochemistry, or preserving synaptic function; neuroimaging modalities exist to measure each of these putative therapeutic goals. In this review, we will focus on imaging biomarkers of potential use in clinical trials of disease-modifying therapies for AD. Before reviewing the imaging studies, however, we will discuss two important concepts that need to be considered in the development of imaging-based markers: clinicopathologic constructs of AD and types of biomarkers.

Constructs of AD: clinical, prodromal, and presymptomatic phases

The pathophysiologic process of AD is thought to take place over years, possibly decades, before the development of dementia. Currently, however, the clinical diagnosis of AD is made after a patient has developed impairment in multiple cognitive domains that is substantial enough to interfere with routine social and/or occupational function (dementia). It is only after this point that FDA-approved medications are currently indicated—that is, clinically probable AD. By this time, substantial neuronal loss and neuropathologic change have damaged many brain regions. Although data from animal models suggest that it may be possible to impede this process as it is developing,[7,8] and potentially reverse some aspects of it,[9] it is not clear whether the pathology typically present when patients are clinically diagnosed with AD can be reversed. Thus, it would be ideal to initiate treatment with neuroprotective medications at a time when—or even before—AD is mildly symptomatic.[10]

To approach this goal, we must improve our capability to identify individuals with prodromal AD—the earliest symptomatic phase of AD before dementia. Currently, individuals are categorized as having mild cognitive impairment (MCI) when symptoms suggestive of AD are present but mild enough that traditional diagnostic criteria (which require functional impairment consistent with dementia) are not fulfilled. This gradual transitional state may last for a number of years. Diagnostic criteria for MCI have been developed[11] and operationalized[12] in a manner that suggests that cohorts of such individuals can be reliably identified for clinical trials. If the pathophysiologic process of AD can be slowed at this stage of the disease, then it may be possible to preserve cognitive function and delay the ultimate development of dementia for a period of time, which is clearly clinically meaningful. Therefore, MCI patients present an excellent target population for clinical trials of disease-modifying therapies.

However, studies of potential imaging-based biomarkers in MCI cohorts face challenges related to clinicopathologic heterogeneity. Although all patients with AD progress through some form of an MCI phase before dementia, the converse is not true. That is, some patients who fulfill MCI criteria may actually have non-AD disease states—pathophysiologic processes other than AD. Furthermore, the rate at which individuals with MCI decline within this diagnostic category and ultimately develop dementia may vary considerably. Thus, although prodromal AD may be identifiable as MCI clinically and it may be possible to reliably operationalize criteria for clinical trials, it is important to recognize the heterogeneity present within this clinical construct. Continued efforts to further refine clinical diagnostic[13] and staging methods[14] should help improve our understanding of the relationships between the characteristics of individuals with MCI and imaging biomarker data.

Finally, presymptomatic AD is the phase of the disease when pathologic alterations are developing but cognitive impairment is not yet apparent. This is likely best studied through the identification of cohorts with particular risk factors, such as genetic determinants [e.g., amyloid precursor protein (APP) or presenilin mutations, Down syndrome] or susceptibility factors [e.g., apolipoprotein E (APOE)-ϵ4]. Ideally, it would ultimately be possible to initiate disease-modifying therapies at this point based on the presence of risk factors, much as is done in the case of primary preventive measures for cerebrovascular disease. Yet given that some of these therapies may not be benign, it would be best to have a panel of biomarkers that could be used to help guide the timing of such therapies, such that individuals at elevated risk for AD could be followed over time. When changes in biomarkers indicate the earliest phase of active pathophysiology, treatment could be initiated.

Types of biomarkers

A recent commentary in this journal,[15] enumerated three aspects of disease pathophysiology in which biomarkers may play important roles: as markers of trait, state, and rate. Neuroimaging-based measures may provide useful data in all of these situations. Disease traits are typically thought of as risk factors, which may involve genetic, anatomic, or physiologic elements, or environmental exposures. A number of imaging studies of cognitively intact individuals at a younger age than the typical onset of AD have begun to investigate anatomic or physiologic differences between those at elevated genetic risk for AD and controls, and to follow these changes over time (e.g., temporoparietal hypometabolism in young APOE-ϵ4 carriers).[16] Ultimately, because most AD traits indicate risk but are not deterministic (i.e., not all APOE-ϵ4 carriers manifest clinical disease), long-term clinical follow-up coupled with the longitudinal assessment of biomarkers will be needed to determine which individuals in these risk groups develop cognitive decline and clinical AD. Thus, biomarkers of disease traits would indicate susceptibility and could potentially be combined with other risk factors to improve the accuracy of risk estimation and prediction of clinical disease.

A measure of disease state enables the detection of clinical or biologic disease in individuals or groups of individuals and is typically thought of as a diagnostic marker. In AD, disease markers are usually considered to reflect the presence of neuropathology, and include measures derived from neuroimaging, serum and CSF, and other biologic materials. However, studies of markers of disease state have been confounded by the notorious difficulty in correlating neuropathologic states themselves with clinical state. That is, patients with clinically probable AD who are equated on clinical metrics may exhibit marked variability in the density and distribution of senile plaques, neurofibrillary tangles, neuronal loss, and other measures of abnormal brain structure. Furthermore, postmortem studies indicate that some cognitively intact individuals, and many MCI patients already carry a heavy burden of AD neuropathology.[17–19] Thus, findings from cross-sectional studies showing that imaging-based markers of brain structure and function in MCI overlap substantially with both cognitively intact older control groups and with AD patient groups are not surprising. These significant individual differences, which confound cross-sectional studies of clinicopathologic state, again reinforce the notion that detailed longitudinal investigations are needed to clarify the relationships between clinical state and measures of neuroanatomy, physiology, and pathology. Some of these types of measures might be useful not only to distinguish patient groups at one point in time, but also to predict risk of future clinical symptoms. As such, these markers provide a window into presymptomatic disease states.

Finally, imaging markers of the rate of disease progression—which allow the tracking of changes over time in pathoanatomic or pathophysiologic alterations in the brain associated with AD—would be particularly useful in evaluating the efficacy of disease-modifying therapeutics. Importantly, although changes in any of these markers may represent changes in the underlying disease process, the rates of change of a marker may or may not correlate with that of other biomarkers or of clinical metrics during the time period of interest. Using imaging-based measures, it may be possible to detect the ability of a putative disease-modifying agent to impede the degenerative process of AD in a shorter period of time than would be necessary to judge slowing of cognitive decline. Whereas validation of biomarkers against clinical outcomes is ultimately essential, as the focus of therapeutics shifts toward prevention or modulation of the neurodegenerative process before the presence of substantial symptoms, such validation takes longer and becomes more difficult. Therefore, the validation of new potential imaging biomarkers (e.g., amyloid imaging, functional MRI) may be performed more efficiently in conjunction with more established imaging markers [e.g., hippocampal volume, fluorodeoxyglucose (FDG)-positron emission tomography (PET)]. Finally, individual variability in rates of decline in MCI and AD is substantial. Imaging-based biomarkers may offer an opportunity for additional power in this regard, which could be gained using a "run-in" phase to quantify subjects' individual rates of change in the imaging marker (e.g., hippocampal volume) before the randomization phase of the clinical trial.

A number of imaging-based biomarkers have begun to show preliminary promise as potential markers of AD traits or of presymptomatic or prodromal disease states. Longitudinal studies of individuals at elevated genetic risk[20–22] and of subjects with mild memory impairment[23–25] suggest that it may be possible to use imaging measures to identify "leveraged cohorts" of individuals who are at elevated risk for a clinical diagnosis of AD within several years. The recruitment of such high-risk leveraged cohorts may be a reasonable strategy for clinical trials of candidate disease-modifying AD therapeutics.

However, as will be reviewed below, the widely ranging initial estimates of sample sizes for clinical trials from these preliminary studies have highlighted the need for further fundamental knowledge regarding the natural history of *in vivo* anatomic and physiologic changes in MCI and AD. Sample size estimates derived from power calculations are influenced primarily by the proposed size of the effect of interest (e.g., difference in rate of atrophy in treated *vs* control patients) and the variance of the data derived from the particular measure used. Im-

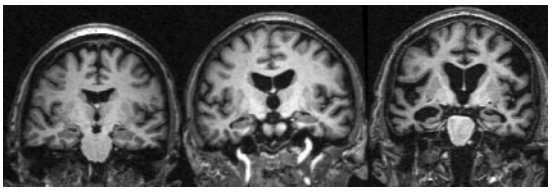

FIG. 1. Coronal MRI sections from individual subjects (Control, MCI, and AD), illustrating mild degree of atrophy in MCI and greater atrophy in mild AD compared with age-matched control.

aging data on which to base estimates of effect sizes for putative disease-modifying therapies for AD have only recently begun to appear in the literature. Variance of the data may be influenced by biologic variability, such as heterogeneity of atrophy rates in the sample, which may relate to age, disease severity, or individual differences in rate of disease progression. Variance is also influenced by measurement variability, which may result from differences in the data acquired between multiple time points (e.g., due to changes in instrumentation or signal acquisition) or in differences in post-scan processing of the data (e.g., selection of regions of interest). Both sources of measurement variability may be compounded by differences between sites in multicenter studies. Such data may be seriously confounded if systematic bias is introduced in any of these sources of variability. Further serial imaging studies examining the natural history of changes in brain structure and function in prodromal AD, as planned in the NIH-funded Alzheimer's Disease Neuroimaging Initiative (ADNI), are essential to gather data that will enable the performance of realistic power calculations for multicenter clinical trials. We will now review some of the specific imaging measures that are under investigation as biomarkers, with a focus on measures that are particularly promising for use in clinical trials of disease-modifying neurotherapeutic agents.

IMAGING BIOMARKERS OF AD-RELATED ALTERATIONS IN BRAIN ANATOMY

Neuroimaging techniques that provide measures of brain structure have been applied to the diagnosis of AD for decades. AD involves the selective degeneration of neuronal populations in specific brain regions starting initially in the medial temporal lobe. This is marked macroscopically by volume loss in these regions. Therefore, in diagnosis or monitoring of progression, neuroimaging measures can be useful in identifying individuals with changes in brain structure consistent with AD (FIG. 1)[26] and in following the degenerative changes in those regions over time. If a drug is able to impede the degenerative process of the disease, it would theoretically slow the rate of atrophy and other changes in brain structure that are hallmarks of the progression of AD.

As MRI and computational technologies have matured,[27] it has become possible to perform increasingly sophisticated investigations of brain morphometry. It is now routine to acquire *in vivo* structural neuroanatomic data for clinical purposes at a resolution of 1 mm^3. In addition, new techniques for the coregistration of one scan to another have improved methods for quantifying structural change over time within individuals. This enables individuals to be used as their own controls, which improves the signal-to-noise ratio of these measures by reducing the noise of individual differences in neuroanatomy that are inherently present in group-comparison studies. MRI measures of brain structure may also be confounded by within-subject variability in hydration status and probably other unknown factors.[28] Despite these caveats, longitudinal MRI measures of changes in brain structure have been successfully used as outcome measures in clinical trials of disease-modifying therapies for multiple sclerosis.[29]

Structural MRI data can be analyzed using traditional manual anatomical methods or semiautomated image processing systems that enable different tissue classes to be distinguished (gray matter, white matter, CSF) and assigned anatomic labels based on probabilistic atlases. Depending on the question of interest, one or more of a variety of methodologic approaches can be used to quantitatively measure aspects of brain structure, including volumes of regions of interest, tissue density, surface area, or topology. Whereas a wide array of approaches have been applied to AD, they can be categorized for our purposes as measures of global brain structure and those of specific regions of interest (ROI). Both of these approaches have been applied cross-sectionally as well as longitudinally to attempt to distinguish AD from other neurodegenerative dementias, and to better understand the natural history of changes in brain structure in normal aging, MCI, and AD.

Global measures of change in brain structure

Methodologic advances in theoretical and practical aspects of computational neuroanatomy have led to the development of sophisticated software to semiautomate the analysis of MR image data.[30] To date, many of these methods provide information on differences in global or large-scale brain structure between groups of individuals or within individuals over time. One such approach is voxel-based morphometry, which involves the spatial normalization of each subject's brain image volume into a common space.[31] Results with this technique have suggested that normal aging is associated with a linear decline in gray matter with relative sparing of medial temporal lobe structures,[32] but that AD patients have widespread atrophy.[33] Another approach, called cortical pattern matching, involves warping individual scan data into a common space, manually delineating major sulcal and gyral landmarks, and then computing statistics that

indicate the discrepancy between the cortical patterns of a study group and those of a control population. This approach and a related hippocampal surface-based method have shown robust capacity to differentiate AD patients from controls, and to quantify change in brain structure over time.[34–36] Finally, one method that has been applied toward the development of surrogate imaging markers for clinical trials is the brain boundary shift integral (BBSI) approach.[37] This semiautomated method involves the rigid coregistration of one scan (baseline) and another (follow-up), and essentially subtracts the follow-up data from the baseline data to calculate the positional shift in global tissue boundaries, thus enabling the quantification of tissue loss at brain-CSF edges over time.

Annual whole brain atrophy rates measured by BBSI are 2–3% in AD patients, whereas they are 0.2–0.5% for normal aging.[37] Rates of whole brain atrophy relate to global decline in cognitive function and cortical neurofibrillary tangles at postmortem examination.[38] The utility of BBSI *versus* manual hippocampal volumetry as biomarkers in clinical trials was compared using a power calculation that estimated that for a drug with an anticipated ability to reduce the rate of cerebral atrophy by 20% (the effect size) over 1 year, the sample size needed in each treatment arm to have 90% power to detect a drug effect would be 207 *versus* 404 patients, respectively.[37] However, these comparisons may not reflect simply methodologic differences as the hippocampal volumetric data used in these calculations were derived from older subjects, who may have greater rates of regional brain atrophy (although this is controversial[39,40]). Similar power calculations based on a semiautomated coregistration technique for tracking change in ventricular size revealed that 135 subjects would be required in each arm to detect a 20% effect in just 6 months[41] Although the BBSI and other heavily automated methods reduce the potential confounds that may occur when individual human operators manually delineate specific neuroanatomic structures,[37] it is possible that drug effects may be best detected by careful measurements of specific ROIs.

Region-of-interest measures of change in brain structure

Quantitative ROI-based MRI methods reliably detect AD-related neuroanatomic abnormalities, with diminished hippocampal volume being the most consistent finding. Reliable protocols have also been developed to detect atrophy of entorhinal cortex and other regions involved in early AD.[24,42] Hippocampal volume derived from MRI correlates strongly with histological hippocampal volume and neuronal loss[43] and severity of AD pathology,[44,45] as well as memory impairment.[46,47] These measures are also useful for the identification of subgroups of individuals with mild memory impairment who will progress to a clinical diagnosis of AD within a few years.[24,48–52] Furthermore, it is possible to detect atrophy of these regions up to 5 years before the expression of clinical symptoms in individuals with APP mutations.[53]

Manual ROI-based longitudinal MRI studies of rates of atrophy in AD have focused primarily on the medial temporal lobe (MTL). The annual rate of hippocampal volume loss is reported to be two to three times greater in mild AD patients than in controls, ranging from 4–8% per year; rates of volumetric change in the temporal lobe and temporal horn of the lateral ventricle also differ significantly between AD patients and controls.[54,55] In a longitudinal study of initially asymptomatic individuals harboring an APP genetic mutation, a hippocampal atrophy rate similar to that of AD patients was detected over the 2 years during which symptoms first appeared.[56]

The potential use of manual volumetric measures as surrogate markers in therapeutic clinical drug trials has recently been investigated. In the largest study, although both MRI and behavioral/cognitive measures changed over time in the expected direction, decline over time was more consistently detected with imaging measures than behavioral/cognitive measures.[57] From these data, the estimated number of subjects per arm required to detect a 50% reduction in the rate of decline over 1 year were: ADAS-Cog, 320; Mini-Mental State Examination (MMSE), 241; temporal horn volume, 54; and hippocampal volume, 21. In a double-blind, placebo-controlled trial of donepezil in patients with AD, the donepezil-treated group demonstrated both the expected symptomatic improvement as well as a lesser degree of hippocampal atrophy than the placebo-treated group over 6 months.[58] The results of these studies support the use of hippocampal volume as a reliable and valid surrogate measure in AD clinical drug trials. However, a recent report from a clinical trial in AD patients using active immunization with β amyloid suggested an increase in atrophy in the patients who developed a significant antibody response, raising the question as to whether a therapeutic response to disease-modifying agents will always parallel reduced rates of atrophy (N. C. Fox, data presented at 9th International Conference on Alzheimer's Disease and Related Disorders, 2004). The explanation for these paradoxical results remains to be elucidated and may ultimately provide valuable information about the relationship of changes measured using structural imaging modalities to the pathophysiology of AD.

Manual ROI-based methods of quantifying changes in brain structure are limited by their operator-dependent, labor-intensive nature, which typically constrains studies to the measurement of a few ROIs. Because the rate of atrophy of different brain regions varies at different stages of AD, this may influence the sensitivity of such measures (e.g., atrophy of entorhinal and hippocampal

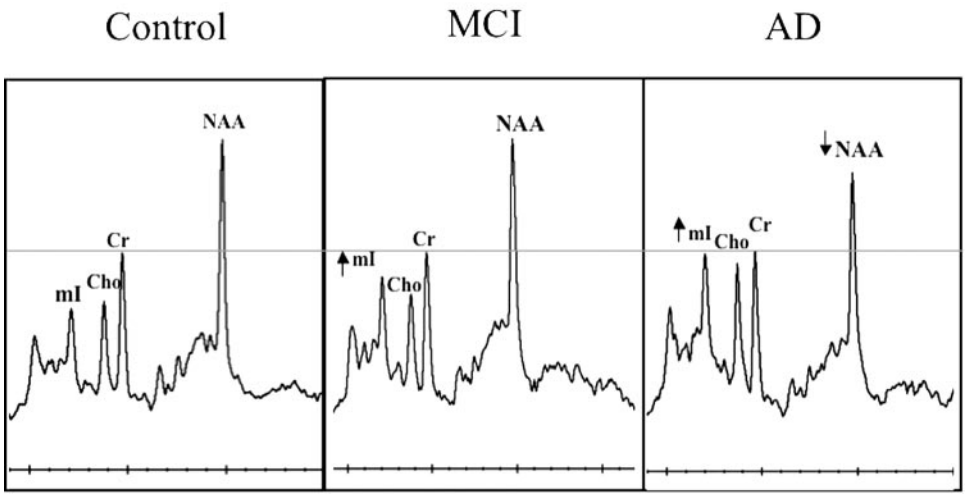

FIG. 2. ^{1}H spectra from posterior cingulate from individual subjects (Control, MCI, and AD), illustrating increased mI peak in MCI and decreased NAA peak in AD. Figure courtesy of Kejal Kantarci, M.D. (Mayo Clinic, Rochester, MN).

regions occur first in MCI,[59] followed by lateral temporal and other neocortical regions). Yet semiautomated computational methods can be used to perform hippocampal volumetry[60] and atlas-based algorithms have been developed to segment the entire brain into subcortical and cortical regions of interest, thus affording more efficient and potentially less biased delineation of a large number of ROIs.[61,62] Other issues that may compromise the reliability of manual morphometric methods include drift in instrument parameters over time and variability in head positioning, both of which may be handled better by automated coregistration techniques. However, manual volumetric measurement approaches can be applied to data that have been "pre-processed" using coregistration methods to correct for these problems. Direct comparison of manual *versus* automated and focal *versus* global methods in a variety of settings will be necessary because it is likely that certain methods will be better suited to particular questions but not others.[60,63,64] Further insights into these questions will be gained from the incorporation of a variety of analytic methods into ongoing large-scale multicenter studies that entail serial MRI scans, such as the Alzheimer's Disease Cooperative Study trial of MCI and the ADNI. These efforts will be supported by the development of collaborative infrastructure through groups such as the Biomedical Informatics Research Network.[65]

A more substantial concern for standard volumetric MRI studies of changes in brain structure is that volume alone does not provide information about tissue composition. That is, neuronal loss may be accompanied by glial proliferation, to which T1-weighted MRI sequences typically used in volumetric studies are not sensitive.[66] If the lack of volumetric change associated with a drug resulted in part from increased glial cell volume rather than the preservation of neurons and their processes, this would likely not be considered a desired outcome. Thus, other types of MR measures that provide information about the biochemical composition of brain tissue may be important methods to include in studies of potential disease-modifying therapies.

IMAGING BIOMARKERS OF AD-RELATED ALTERATIONS IN BRAIN CHEMISTRY

As reviewed separately in the current volume,[66] proton magnetic resonance spectroscopic imaging (MRS) is a technique that enables the quantification of particular neurochemical constituents of brain tissue. Metabolic components that are commonly measured include N-acetyl aspartate (NAA) and myoinositol (mI), which are thought to represent the density of living neurons and glial cells, respectively, and choline (Cho), which is a marker of cell membrane turnover. In AD patients, decreases in NAA are found in MTL, posterior cingulate (FIG. 2), and other regions typically affected by neurofibrillary pathology early in the disease.[67,68] Levels of mI tend to be increased in AD, and Cho may be increased or unchanged. In individuals with MCI, MRS measures are different from normal aging[68] and relate to memory performance;[69] these cross-sectional studies suggest that mI may increase before decreases in NAA and Cho. The combination of volumetric and spectroscopic MR measures appears to provide better diagnostic sensitivity and specificity for AD (*v*s controls) than either measure alone.[70]

Several longitudinal clinical-imaging studies have examined changes in MRS with progression of AD. Over the course of a year, total gray-matter NAA declined to a greater degree in AD patients (12.36%) than controls (0.94%), correlated with decline on a clinical measure of global impairment, and was more sensitive than change

in total gray-matter volume.[71] In AD patients, 2-year decline in MTL NAA correlated with decline on MMSE[72]; a similar though nonsignificant finding was observed after 1 year.[73] Longitudinal changes in MRS measures have also been evaluated in AD patients during therapeutic trials with cholinergic agents.[58,74,75] Changes in both NAA and Cho correlate with cognitive function. These findings not only support the feasibility of MRS measures in AD clinical trials, but also indicate that AD-related changes detected by MRS may be reversible, and may reflect aspects of neuronal integrity or function. Thus, spectroscopic measures in AD may provide a bridge between traditional measures of brain structure and function.

IMAGING BIOMARKERS OF AD-RELATED ALTERATIONS IN BRAIN FUNCTION

Techniques to measure aspects of brain function *in vivo* have begun to provide revolutionary insights into cerebral activity at rest, during task performance, and the alterations that occur in individuals with neurodegenerative disease. Because functional neuroimaging tools assess inherently dynamic processes that may change over short time intervals in relation to a host of factors, these measures have unique characteristics that may offer both strengths and weaknesses as potential biomarkers for neurodegenerative disease. Functional neuroimaging measures may be affected by transient brain and body states at the time of imaging, such as arousal, attention, sleep deprivation, sensory processing of irrelevant stimuli, or the effects of substances with pharmacologic CNS activity. Imaging measures of brain function may also be more sensitive than structural measures to constitutional or chronic differences between individuals, such as genetics, intelligence or educational level, learning, mood, or medication use. Whereas these may be effects of interest in certain experimental settings, they need to be controlled when the focus is on changes in disease over time and the goal is to optimize biomarker test-retest reliability. Functional neuroimaging data can be categorized as measures obtained "at rest" or during task performance.

Brain metabolism and perfusion at rest: FDG-PET and single-photon emission computed tomography

Functional tomographic techniques detect signals related to functional properties of brain regions three-dimensionally using radiolabeled compounds. The two major techniques that have been applied to AD are PET and single-photon emission computed tomography (SPECT). Using PET, the regional cerebral metabolic rate of glucose can be measured (with FDG). With SPECT, cerebral blood flow rates (perfusion) can be measured. For over 20 years, these techniques have been applied to the study of dementia, and one of the most consistent findings in AD is a reduction at rest of metabolism and perfusion in posterior temporo-parietal, posterior cingulate, and frontal regions, with sparing of primary somatomotor cortices (FIG. 3). Animal model studies have also suggested that posterior cingulate hypometabolism may be an early feature of the disease,[76] and demonstrated that it occurs after entorhinal lesions, possibly as a result of disconnection.[77] This functional signature of AD has been studied extensively as a potential marker of disease state—a diagnostic marker to differentiate AD from normal aging and other neurodegenerative diseases, and can do so in the proper clinical context with relatively high sensitivity and specificity when compared with clinical diagnoses.[78] In PET or SPECT studies of AD patients followed to autopsy, the *in vivo* resting functional findings have also demonstrated relatively high sensitivity to detect postmortem AD neuropathology but somewhat lower specificity.[79–81] In addition, multicenter studies have demonstrated that PET data acquired using different instruments can be pooled in a manner that minimizes site-related variance and enables the detection of disease effects.[82,83] These findings have been borne out by an international multicenter collaborative group that pooled PET and pathologic data from 138 patients who had undergone dementia evaluations and been longitudinally followed at centers around the world.[84] Although the functional neuroimaging measures were diagnostically sensitive, specific, and useful for prediction of cognitive course and pathologic outcome, their limitations should be kept in mind. PET and SPECT findings can be

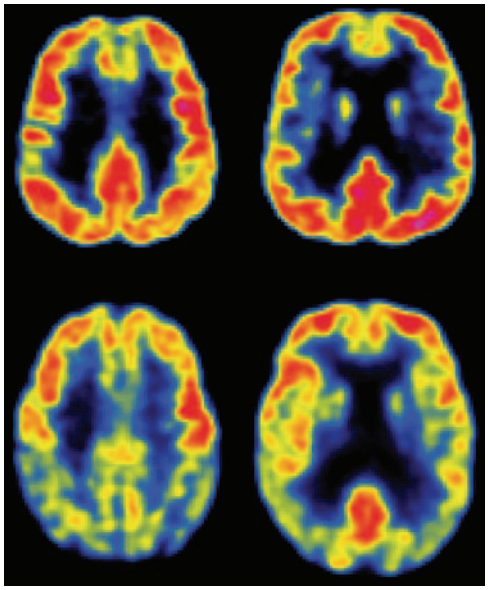

FIG. 3. FDG-PET data from an older control subject (top) and a patient with probable Alzheimer's disease (bottom), illustrating prominent temporoparietal hypometabolism. Figure courtesy of Keith Johnson, M.D. (Massachusetts General Hospital, Boston, MA).

affected by subject age,[85] analytic methods,[86] and atrophy.[87] A more complete overview of PET and SPECT including technological and clinical aspects has been provided separately in the current volume.[88,89]

Longitudinal studies have shown that baseline PET and SPECT measures are useful for the prediction of future cognitive decline in AD patients[90,91] and the early detection of disease state in individuals with MCI.[23,82,92,93] Serial functional imaging studies have demonstrated that progressive metabolic decline correlates with cognitive decline in AD patients.[94,95] Power calculations suggest that PET measures may be more sensitive than cognitive measures in a 1-year clinical drug trial, with estimates of 41–228 and 246–390 subjects, respectively (effect size = 25%, power = 80%).[96]

Tantalizing results are emerging from longitudinal studies with serial FDG-PET measures in subjects at elevated risk for clinical AD, but in whom symptoms are very mild or absent. Progressive metabolic abnormalities parallel cognitive decline in both older cognitively intact individuals[97] and subjects with mild memory impairment who carry the APOE-ϵ4 allele.[86] In individuals in their fifties without cognitive decline, progressive metabolic decline has been observed in ϵ4 carriers after 2 years.[98] For a 2-year treatment study of these higher-risk asymptomatic individuals, a sample size of 50–115 subjects per treatment arm would be needed to detect a drug effect (effect size = 25%, power = 0.8).

Finally, PET and SPECT measures of resting brain function appear to be sensitive to medication effects in clinical drug trials and relate to clinical measures in a manner that suggests their potential utility as surrogate markers. In four studies of cerebral metabolism or perfusion in AD patients given cholinesterase inhibitors, these functional brain measures parallel clinical measures in demonstrating stability or improvement in treated *versus* placebo groups or in predicting response in treated patients.[99–102]

Task-related brain hemodynamics and metabolism: functional MRI and FDG-PET

Functional neuroimaging techniques can also be used to measure regional brain "activation" during the performance of cognitive tasks. Most such techniques, including FDG-PET, O-15-PET, and functional MRI (fMRI), measure task-related changes in regional brain metabolism, blood flow, or deoxyhemoglobin concentrations, which are thought to reflect neuronal activity. Functional neuroimaging studies have shown that AD patients differ on these measures of regional brain activation from controls during the performance of a variety of types of tasks. In fMRI studies of memory task performance in patients diagnosed with AD, hippocampal and parahippocampal activation is consistently decreased in comparison to older controls (FIG. 4).[103–107] Whereas memory-

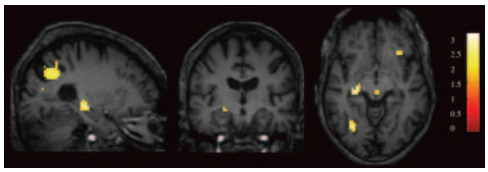

FIG. 4. Decreased medial temporal lobe activation can be detected during the performance of memory tasks in mild AD patients compared with nondemented older individuals, as measured by fMRI. Group statistical comparison showing regions with decreased activation in AD patients compared to age-matched normal controls.

task related fMRI data regarding MTL activation in individuals with MCI are less consistent, with reports of both decreased and increased activation,[25,103,107] they do indicate that differences are present in comparison to older controls. Some of the variability in fMRI data on MTL activation appears to relate to degree of impairment along the spectrum of MCI, which suggests that fMRI may be sensitive to relatively subtle clinical differences.[25] Moreover, differences in memory-related MTL activation are associated with likelihood of subsequent cognitive decline,[25] which implies that fMRI may be a sensitive technique for prediction of future clinical status. As trait biomarkers, functional neuroimaging techniques can detect brain activation differences between individuals at elevated genetic risk for AD and controls,[21,22] which may potentially be markers of presymptomatic disease.

FMRI may be particularly valuable in evaluating acute and subacute effects of medications on neural activity that may have both symptomatic and disease-modifying properties. Recent animal studies have suggested that antiamyloid strategies may result in acute changes in synaptic function and behavior, in addition to altering amyloid plaque formation.[108,109] FMRI studies have demonstrated significant alterations in memory-related activation with the administration of pharmacologic agents known to impair memory.[110] Finally, fMRI investigations of the effects of cognitive enhancing drugs on brain activation during cognitive task performance have shown that changes can be detected after administration of cholinesterase inhibitors in patients with AD[111] and MCI.[112]

A caveat essential to the interpretation of task-related functional neuroimaging data is that healthy individuals of any age demonstrate differences in brain activation depending on how well they are able to perform the particular task. For example, when cognitively intact individuals learn new information during fMRI scanning, the strength of this signal is related to subsequent ability to remember the information.[113–117] AD patients typically perform less well on the memory tasks, which complicates the interpretation of these data.[118] Yet MCI and AD patients may recruit additional brain tissue or regions than controls during task performance,[25,119,120]

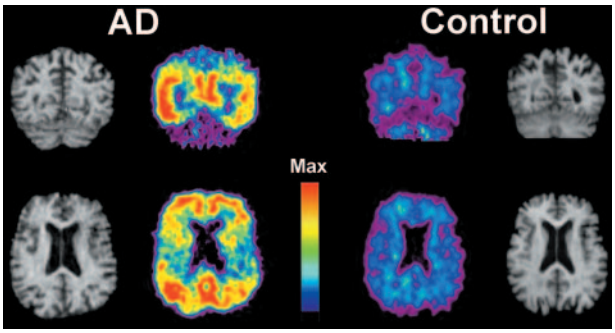

FIG. 5. *In vivo* PET-based detection of β amyloid. Increased retention of Pittsburgh compound-B (PIB) is found in frontal and temporo-parietal regions in patients with clinical AD. Figure courtesy of William E. Klunk, M.D., Ph.D. (University of Pittsburgh Medical Center, Pittsburgh, PA).

as has been seen in patients with other neurologic disorders.[121] The recruitment of additional brain regions during task performance by patients with neurodegenerative or other neurologic disease may indicate the presence of processes attempting to compensate for damaged networks.[25,122] While the task performance factor is important to consider when designing or interpreting functional neuroimaging studies of MCI or AD, it also indicates that these imaging biomarkers may be particularly sensitive to changes in cognitive function, which not only provides face validity for these measures but also supports their potential use in short-term, early proof-of-concept drug trials.

IMAGING BIOMARKERS OF AD-RELATED BRAIN PATHOLOGY

Extensive efforts to develop *in vivo* methods to more directly measure AD neuropathology have begun to bear fruit. Much of this work has been directed toward the development of tracers that can be safely administered to humans to label proteins associated with the classic neuropathologic findings in AD. The majority of these compounds are thought to label fibrillary β amyloid, but some reports have reported labeling of τ-based pathology as well. Tracers are in development that can be detected using PET, SPECT, and even MRI. The first reports of clinical applications of these tracers have only recently appeared in the literature (FIG. 5).[123,124]

The successful visualization of direct markers of neuropathology in living humans is a major step forward in the field and suggests that more specific *in vivo* diagnostic and monitoring capabilities may be on the horizon. In addition, these approaches may be very useful in the burgeoning efforts to improve translational research between animal models and humans. However, a number of issues will need to be addressed as part of the validation of these methods as surrogate markers. Whereas visualization of a signal of pathology has been demonstrated, work is still in progress to refine quantitative metrics and determine the specificity of these measures. Finally, it is not yet clear how early in prodromal or presymptomatic AD these imaging pathologic signals will be detectable.

CONCLUSIONS

As a variety of imaging biomarkers of anatomy, chemistry, physiology, and pathology in AD become available, these tools may be employed in a targeted manner in studies of putative neurotherapeutics. Substantial progress has already been made in validating a number of imaging biomarkers of AD against clinical and pathologic data, and several potential roles for imaging markers in drug development are emerging. Preliminary comparisons of imaging measures to standard cognitive or behavioral measures in clinical trials suggest that at least some types of imaging measures show changes that are expected in AD over time more consistently than behavioral measures.[57] In large, multicenter phase 3 studies of drug efficacy, the use of an imaging-based outcome measure may be more reliable than standard clinical or cognitive outcome measures, thereby increasing power to detect a small effect and reducing sample size. Conversely, an imaging-based measure (e.g., hippocampal atrophy or temporoparietal hypoperfusion) could be used along with clinical and psychometric measures as inclusion criteria to reduce heterogeneity of subjects and select a "leveraged cohort" of individuals who have a greater likelihood of a given clinical outcome, such as cognitive decline or "conversion" from MCI to AD within a few years (e.g., high risk of imminent diagnosis of clinical AD due to genetic risk or clinical characteristics *plus* hippocampal atrophy or temporoparietal hypofunction). Such applications of imaging-based biomarkers in large-scale multicenter clinical trials with hundreds of subjects may necessitate "high throughput" markers—those that can be derived from standardized, efficient data acquisition and processing tools. In early phase studies, in which a "go ahead" or "kill" decision for a new compound hinges on a pivotal, proof-of-concept trial, it may be acceptable to use a relatively novel, more labor-intensive, less widely available, or less cost-effective imaging-based measure if it has the capacity to detect evidence of the presence or absence of a disease-modifying effect in a short timeframe or with few subjects. For example, studies of AD animal models have begun to suggest that acute treatment with β-amyloid antiaggregating compounds or monoclonal antibodies may rapidly rescue long-term potentiation,[109] and functional neuroimaging techniques can clearly reveal changes in brain activity associated with the acute administration of psychopharmacologic agents.[110,111] It is intriguing to imagine a potential mouse-to-man transla-

tional drug development pipeline in which drugs that show promise of safety and efficacy in animal models are quickly taken to proof-of-concept human trials. Such trials might involve the use of a battery of neuroimaging markers as part of the inclusion criteria to select asymptomatic or mildly symptomatic patients with a particular level of AD pathology.

Finally, imaging measures also hold promise for predicting future change in clinical status or cognitive performance, and for detecting abnormalities in brain structure or function in cognitively intact individuals before symptom onset. Thus, imaging biomarkers may take on even greater importance as potential surrogate markers if treatments are initiated earlier in the disease, when symptoms are very mild or absent and thus difficult to use as outcome measures. If we are to achieve the goal of identifying disease-modifying therapies to delay the clinical symptoms of AD in cognitively intact individuals at elevated risk for the disease, we will need validated surrogate markers of disease pathophysiology. The rapid pace of developments in the field of imaging biomarkers and their preliminary use in clinical trials provides reason for optimism that progress is being made on both fronts.

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