Amyloid negativity in patients with clinically diagnosed Alzheimer disease and MCI

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ABSTRACT

Objective: To examine the clinical and biomarker characteristics of patients with amyloid-negative Alzheimer disease (AD) and mild cognitive impairment (MCI) from the Alzheimer's Disease Neuroimaging Initiative (ADNI), a prospective cohort study.

Methods: We first investigated the reliability of florbetapir− PET in patients with AD and patients with MCI using CSF-$A\beta_{1-42}$ as a comparison amyloid measurement. We then compared florbetapir− vs florbetapir+ patients with respect to several AD-specific biomarkers, baseline and longitudinal cognitive measurements, and demographic and clinician report data.

Results: Florbetapir and CSF-$A\beta_{1-42}$ +/− status agreed for 98% of ADs (89% of MCIs), indicating that most florbetapir− scans were a reliable representation of amyloid status. Florbetapir− AD (n = 27/177; 15%) and MCI (n = 74/217, 34%) were more likely to be *APOE*4-negative (MCI 83%, AD 96%) than their florbetapir+ counterparts (MCI 30%, AD 24%). Florbetapir− patients also had less AD-specific hypometabolism, lower CSF p-tau and t-tau, and better longitudinal cognitive performance, and were more likely to be taking medication for depression. In MCI only, florbetapir− participants had less hippocampal atrophy and hypometabolism and lower functional activity questionnaire scores compared to florbetapir+ participants.

Conclusions: Overall, image analysis problems do not appear to be a primary explanation of amyloid negativity. Florbetapir− ADNI patients have a variety of clinical and biomarker features that differ from their florbetapir+ counterparts, suggesting that one or more non-AD etiologies (which may include vascular disease and depression) account for their AD-like phenotype.
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GLOSSARY

Aβ = β-amyloid; **AD** = Alzheimer disease; **ADAS-cog** = Alzheimer's Disease Assessment Scale–cognitive subscale; **ADNI** = Alzheimer's Disease Neuroimaging Initiative; **AGD** = argyrophilic grain disease; **MCI** = mild cognitive impairment; **metaROI** = previously validated region of interest; **MMSE** = Mini-Mental State Examination; **MPRAGE** = magnetization-prepared rapid gradient echo; **RAVLT** = Rey Auditory Verbal Learning Test; **SUVR** = standardized uptake value ratio; **TBM-SyN** = tensor-based morphometry-symmetric diffeomorphic image normalization method; **VBM** = voxel-based morphometry; **WM** = white matter.

The rate of β-amyloid (Aβ) negativity in clinically diagnosed Alzheimer disease (AD) varies across a variety of study populations and as a function of *APOE* genotype status.[1–6] Previous studies of patients with clinically diagnosed AD have shown that 12% were negative on amyloid PET in a recent meta-analysis,[7] and 10%–25% of *APOE*4-negative patients with AD did not meet the neuropathologic criteria for AD at autopsy.[8,9] Older adults with an amnestic profile that is suggestive of AD comprise a diverse group with heterogeneous pathology. Hippocampal sclerosis, argyrophilic grain disease, vascular dementia, Lewy body disease, and frontotemporal dementia have been observed at autopsy in addition to AD pathology[10] and in Aβ− cases with an antemortem AD diagnosis.[9]

Supplemental data at Neurology.org

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Coinvestigators are listed on the *Neurology*® Web site at Neurology.org.

Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

The goal of this study was to compare patients with Aβ− mild cognitive impairment (MCI) and patients with AD enrolled in the Alzheimer's Disease Neuroimaging Initiative (ADNI) with their Aβ+ counterparts on a number of clinical, neuropsychological, and biomarker characteristics. ADNI was designed as a model for AD clinical trials, so the diagnostic accuracy and Aβ status of these patients should be reasonably representative of those expected to enroll in clinical trials.

We first examined the extent to which image analysis problems may account for some florbetapir− cases using CSF Aβ measurements and reliability of image analysis. Next, we compared Aβ− and Aβ+ patients with AD and patients with MCI on baseline and longitudinal measurements that are sensitive to AD, including clinical evaluations, *APOE4* status, FDG-PET and structural MRI, and cognitive performance.

METHODS

Participants. ADNI is a multisite longitudinal biomarker study that has enrolled over 1,500 cognitively normal older individuals, people with early or late amnestic MCI, and people with early AD (www.adni-info.org).

As of June 2015, there were 177 patients with AD and 217 patients with (late) MCI with at least a baseline florbetapir scan and *APOE4* genotyping. MCI and AD diagnoses were made using standard criteria that have been reported previously.[11]

The amount of data available at the time of this study (August 2015) varies across measurements (table). Concurrent clinical, cognitive, and FDG-PET data were available for ≥90% of participants, while concurrent CSF and hippocampal volume data were available for ≥70% of participants. More than one postbaseline follow-up clinical visit was available for at least 90% of participants. A 2-year follow-up florbetapir scan was available in 50% of participants.

Standard protocol approvals, registrations, and patient consents. All participants gave written informed consent that was approved by the Institutional Review Board of each participating institution.

Florbetapir PET image processing. Florbetapir synthesis and image acquisition details are described in detail elsewhere.[12] Briefly, florbetapir images consisted of 4 × 5 minute frames acquired at 50–70 minutes postinjection, which were realigned, averaged, resliced to a common voxel size (1.5 mm^3), and smoothed to a common resolution of 8 mm^3 full width at half maximum. Magnetization-prepared rapid gradient echo (MPRAGE) images that were acquired concurrently with the baseline florbetapir images were used as a structural template to define cortical and reference regions in native space for each participant using Freesurfer (v4.5.0) as described previously.[12–14]

Florbetapir scans for each participant were coregistered to baseline structural MRI scans, which were subsequently used to extract weighted cortical retention means (standardized uptake value ratios [SUVRs]) from frontal, cingulate, parietal, and temporal regions that were averaged and divided by a whole cerebellum reference region to create a SUVR with a positivity threshold of 1.11 as described in greater detail elsewhere[12,13] and online.[15] We also investigated several alternative reference regions and applied positivity thresholds that were derived using a linear transformation of the whole-cerebellum normalized SUVRs as described previously[16] (cerebellar gray matter, 1.26; brainstem, 0.79; subcortical white matter [WM] eroded away from cortex,[17] 0.62; and a composite region made up of brainstem, whole cerebellum, and eroded WM, 0.79).

FDG-PET image processing. FDG image data were acquired 30–60 minutes postinjection, and fully preprocessed images were downloaded from the ADNI Web site (adni.loni.usc.edu). We then spatially normalized each FDG image to the standard 15O-H_2O PET template using SPM5 and extracted mean FDG uptake for each participant from a set of study-independent and previously validated regions of interest (metaROIs) located in right and left inferior temporal and lateral parietal regions, and a bilateral posterior cingulate cortex region relative to the mean of a pons and cerebellar vermis reference region.[18]

CSF analysis. CSF $A\beta_{1–42}$, t-tau, and p-tau measurements were acquired concurrently with florbetapir scans at baseline and analyzed by the ADNI Biomarker core laboratory. We applied autopsy-validated CSF $A\beta_{1–42}$, t-tau, and p-tau autopsy-validated positivity cutoffs of 192 pg/mL, 93, and 23 to determine positivity as described previously.[19]

Structural MRI analyses. Cross-sectional structural differences were assessed using hippocampal volumes defined on MPRAGE images by Freesurfer v5.1 (v4.3 for ADNI1 continuing participants) and divided by total intracranial volume to adjust for head size. We observed no differences in data analyzed with Freesurfer versions. Structural change over time was measured using a summary score developed by the Mayo Clinic that represents change between pairs of scans in 31 AD-specific regions of interest, known as the tensor-based morphometry-symmetric diffeomorphic image normalization method (TBM-SyN).[20] The average available follow-up time for longitudinal structural MRI scans was 1.3 ± 0.5 years. White matter hyperintensity volumes at baseline as percent of intracranial volume were calculated using coregistered fluid-attenuated inversion recovery and MPRAGE images as described previously.[21]

Clinical and cognitive measurements. We examined several clinical and cognitive performance measurements including baseline and longitudinal performance on the Mini-Mental State Examination (MMSE),[22] the Functional Assessment Questionnaire,[23] the Rey Auditory Verbal Learning Test (RAVLT),[24] and Alzheimer's Disease Assessment Scale–cognitive subscale (ADAS-cog).[25] We also examined baseline scores on the Geriatric Depression Scale[26] although this test was used as a screening tool for ADNI enrollment; participants with a score higher than 5 at baseline were excluded. The average available follow-up time for longitudinal cognitive measurements was 1.4 ± 0.8 years.

We also examined the following dichotomous variables in order to identify the extent to which the clinical profile was consistent with AD: whether conversion from MCI to AD occurred during the 1.4 ± 0.8 years clinical follow-up (patients with MCI only), whether there was a family history of dementia or AD, whether a history of hypertension was present, whether the patient was taking medication for depression or for symptoms of AD (acetylcholinesterase inhibitors) at baseline, and whether the clinician evaluating the patient reported that symptoms were possibly, as opposed to probably, due to AD (patients with AD only).

Table Patients with florbetapir-negative and -positive mild cognitive impairment (MCI) and Alzheimer disease (AD) compared across a variety of demographic, clinical, and biomarker variables

	Late MCI (n = 217)		AD (n = 177)		
	Florbetapir−	Florbetapir+	Florbetapir−	Florbetapir+	Available data, %
Demographics					
No. (%)	74 (34)	143	27 (15)	150	
Age, y	74.4 (9.3)	7,424 (7.5)	78.0 (7.3)[a]	74.4 (8.0)[a]	
Sex, % female	0.43	0.43	0.18[b]	0.45[b]	
Education, y	16.4 (2.6)	16.2 (2.9)	16.6 (2.2)	15.8 (2.7)	
Clinical					
Conversion from MCI to AD	0.11[c]	0.45[c]			
Functional Assessment Questionnaire	2.5 (4.0)[c]	4.6 (4.9)[c]	14.1 (7.8)	14.0 (7.0)	>95
Geriatric Depression Scale	1.8 (1.8)	2.0 (1.8)	3.0 (3.5)[b]	1.6 (1.6)[b]	>95
Depression medication use	0.31[a]	0.14[a]	0.22[a]	0.07[a]	>95
AD medication use	0.11[c]	0.34[c]	0.41	0.45	>95
Family history dementia	0.59	0.59	0.44	0.61	>95
Family history AD	0.34	0.3	0.26	0.33	>95
Possible (not probable) AD			0.19[c]	0.01[c]	>95
Hypertension	0.45	0.45	0.63[b]	0.41[b]	>95
Biomarkers					
*APOE*4+, %	0.16[c]	0.71[c]	0.04[c]	0.75[c]	
FDG metaROI	1.28 (0.11)[c]	1.20 (0.13)[c]	1.18 (0.14)[c]	1.04 (0.15)[c]	>95
FDG metaROI+, %	0.27[c]	0.55[c]	0.68[a]	0.88[a]	>95
HippVol	0.005 (0.001)[a]	0.004 (0.001)[a]	0.004 (0.001)	0.004 (0.001)	>70
CSF Aβ	215 (53)[c]	137 (28)[c]	215 (54)[c]	127 (21)[c]	>70
CSF Aβ+, %	0.27[c]	0.96[c]	0.26[c]	1[c]	>70
CSF p-tau	29 (14)[c]	55 (28)[c]	34 (15)[c]	63 (34)[c]	>70
CSF p-tau+, %	0.59[c]	0.94[c]	0.68[c]	0.99[c]	>70
CSF t-tau	59 (29)[c]	118 (55)[c]	94 (51)[a]	137 (65)[a]	>70
CSF t-tau+, %	0.16[c]	0.63[c]	0.42[a]	0.74[a]	>70
WM hyperintensities (% of ICV)	0.44 (0.51)	0.56 (0.75)	0.35 (0.27)	0.59 (0.65)	>70
Longitudinal biomarkers					
Longitudinal florbetapir, % pos 2 y	0.07[a]	0.98[a]	0.11[a]	1[a]	50
TBM-SyN slope	−0.01 (0.01)[c]	−0.02 (0.02)[c]	−0.02 (0.02)[a]	−0.04 (0.03)[a]	60
Baseline cognitive					
MMSE	28.3 (1.5)[c]	27.2 (2.0)[c]	22.6 (3.7)	22.6 (3.2)	>95
ADAS-cog	9.1 (4.1)[c]	12.0 (5.3)[c]	18.4 (7.7)[b]	21.5 (8.2)[b]	>95
RAVLT free recall	36.1 (10.7)	31.5 (9.7)	23.9 (9.9)	21.9 (7.2)	>95
Longitudinal cognitive					
MMSE slope	−0.4 (1.1)[c]	−1.4 (1.9)[c]	−1.0 (3.4)[a]	−2.8 (3.7)[a]	>90
ADAS-cog slope	0.1 (1.8)[c]	2.5 (3.7)[c]	0.5 (6.1)[a]	5.2 (7.9)[a]	>90
RAVLT slope	−0.4 (4.3)[c]	−2.6 (4.6)[c]	−2.8 (5.4)	−3.7 (6.0)	>90

Abbreviations: Aβ = β-amyloid; ADAS-cog = Alzheimer's Disease Assessment Scale–cognitive subscale; ICV = intracranial volume; metaROI = previously validated region of interest; MMSE = Mini-Mental State Examination; RAVLT = Rey Auditory Verbal Learning Test; TBM-SyN = tensor-based morphometry-symmetric diffeomorphic image normalization method; WM = white matter.
Mean (SD) shown for continuous variables and proportion positive/abnormal shown for dichotomous variables. The statistical significance of the florbetapir negative vs positive comparison for each group is shown.
[a] $0.001 < p < 0.05$.
[b] $0.05 \leq p \leq 0.10$.
[c] $p \leq 0.001$.

Statistical methods. For each longitudinal cognitive and TBM-SyN measurement, a slope was calculated for each participant. Because of the discrepancy in florbetapir+/− group sizes and the nonparametric distributions of some variables of interest, we carried out the Mann-Whitney U test at $\alpha = 0.05$ to evaluate differences in continuous variables between the florbetapir+/− MCI and AD groups. Group differences in dichotomous variables were evaluated using the χ^2 test.

We carried out voxelwise FDG-PET and voxel-based morphometry (VBM) analyses for participants with available whole-brain data (>95% of participants). Voxelwise spatially normalized and FDG-PET data were intensity normalized using the pons/vermis reference region. An independent-samples t test contrasting florbetapir+ vs florbetapir− patients was carried out at $p < 0.001$ uncorrected for the AD and MCI groups in SPM8, with age, sex, and education as nuisance covariates. To examine whole-brain structural differences, we performed the same contrast using VBM with the DARTEL toolbox in SPM8.[27]

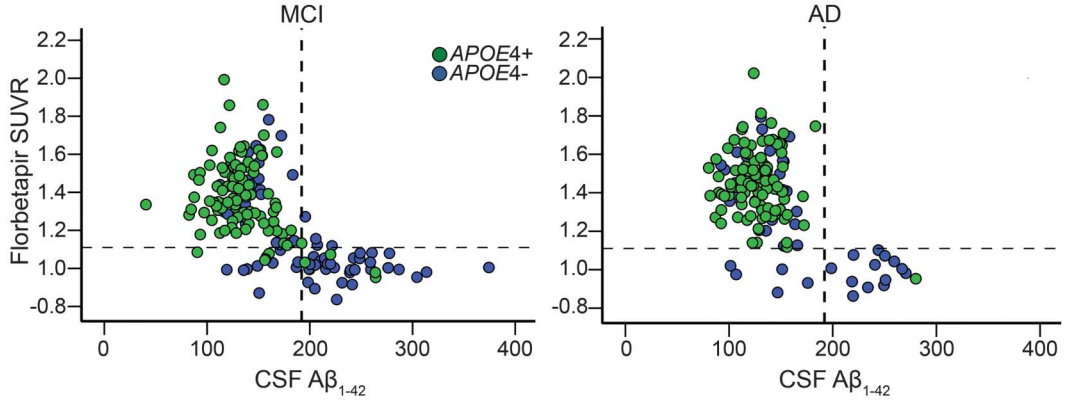
RESULTS **PET technical factors.** Comparing amyloid status between whole cerebellum reference and 4 alternative reference regions (cerebellar GM, white matter, brainstem, composite), we found that status changed in 0%–15% of patients with florbetapir− AD and in 1%–12% of patients with florbetapir− MCI.

For participants with an available 2-year follow-up florbetapir scan (approximately 50% of the sample), we also examined change in amyloid status using the whole cerebellum reference region. The test-retest reliability of the SUVRs was approximately 1.2% ± 0.8% (absolute mean change and SD in stable amyloid-negative controls), as reported previously.[17] Ninety-eight percent of patients with MCI and 100% of patients with AD who were florbetapir+ at baseline remained positive. Four of 54 (7%) patients with MCI and (1/9) 11% of patients with AD who were florbetapir− at baseline were positive at follow-up.

The relationship between florbetapir and CSF $A\beta_{1–42}$ measurements by *APOE*4 status is shown for ADs and MCIs in figure 1 (upper left quadrant, both abnormal; lower right, both normal). A total of 5/137 (4%) patients with AD and 18/170 (11%) patients with MCI had discordant CSF $A\beta_{1–42}$ and florbetapir measurements (upper right and lower left quadrants), and the majority of these (100% of discordant AD and 72% of discordant MCI) were CSF $A\beta_{1–42}$+/florbetapir−. Of florbetapir− cases only, about 3/4 of participants from each diagnostic group were also negative on CSF $A\beta_{1–42}$ (14/19 or 74% of AD and 36/49 or 74% of MCI). CSF Aβ− participants were also disproportionately *APOE*4−. Of the 14 patients with AD and 36 patients with MCI who were negative on both CSF $A\beta_{1–42}$ and florbetapir, 13/14 (93%) and 32/36 (89%) were *APOE*4−.

Comparison of florbetapir+/− groups. ***Demographic and clinical variables.*** Demographic, clinical, cognitive, and biomarker characteristics of the florbetapir+/− MCI and AD groups are summarized in table. In AD only, florbetapir− patients were older than florbetapir+ patients and marginally more likely to be male. On clinical evaluation variables, MCI florbetapir− patients had lower Functional Assessment Questionnaire scores (less functional impairment) and were less likely to convert to AD and to be taking medication for AD (acetylcholinesterase inhibitors). Florbetapir− patients in both groups were more likely to be taking medication for depression, and in AD, florbetapir− patients had marginally higher depression scores and were marginally more likely to have a history of hypertension. Clinicians were more likely to rate the symptoms of florbetapir− AD patients as possibly (rather than probably) due to an AD diagnosis.

Figure 1 **Agreement between CSF $A\beta_{1-42}$ and florbetapir− PET**



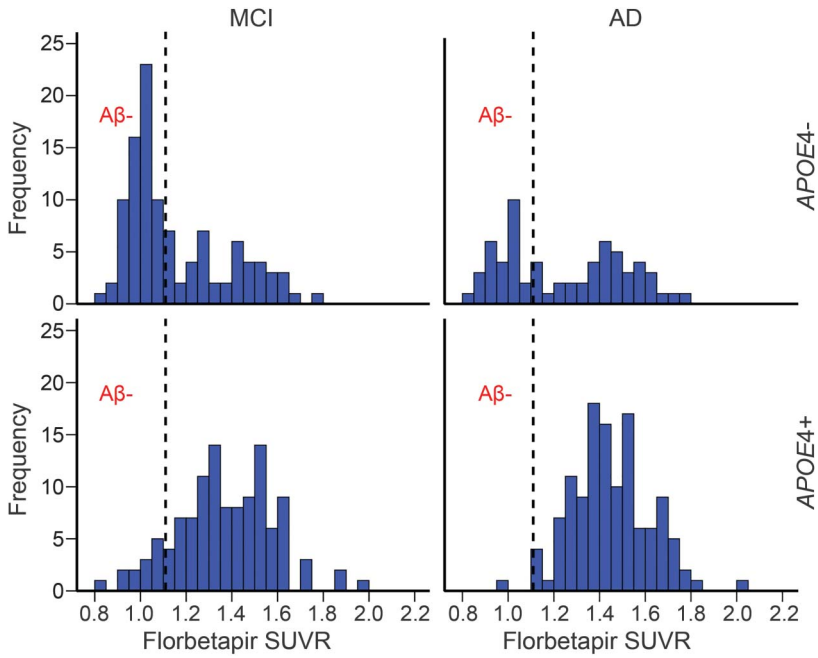
The relationship between concurrent cortical summary florbetapir standardized uptake value ratios (SUVRs) and available CSF $A\beta_{1-42}$ measurements is shown for the mild cognitive impairment (MCI) and Alzheimer disease (AD) groups (*APOE*4+ participants = green, *APOE*4− participants = blue). Dotted lines represent positivity thresholds for each measure (see Methods).

Biomarkers. As shown in figure 2, the majority of florbetapir− patients were *APOE4*− (MCI: 84% *APOE4*−; AD: 96% *APOE4*−) whereas florbetapir+ patients showed the opposite pattern (MCI: 30% *APOE4*−; AD: 24% *APOE4*−) (figure 2). The florbetapir− groups had less hypometabolism in characteristic AD (metaROI) regions, had lower CSF t-tau and p-tau, and had less longitudinal atrophy in AD-specific (TBM-SyN) regions (table). MCI florbetapir− patients (but not AD) had less hippocampal atrophy (table). Groups did not differ on white matter hyperintensity volume.

Cognitive performance. Patients with florbetapir− MCI had better baseline performance than florbetapir+ patients on MMSE, ADAS-cog, and RAVLT, while patients with florbetapir− AD were marginally less impaired on ADAS-cog but did not differ on RAVLT or MMSE. Longitudinally, patients with florbetapir− MCI and AD declined more slowly than florbetapir+ patients on the ADAS-cog (figure 3) and the MMSE, and patients with florbetapir− MCI also declined more slowly on the RAVLT.

Voxelwise comparisons. Because we observed FDG-PET and structural MRI effects in AD-specific regions (see table), we carried out voxelwise FDG-PET and structural MRI analyses to determine if the florbetapir+/− groups differed across a broader set of regions. As shown in figure 4A, patients with florbetapir− MCI and AD had less hypometabolism than florbetapir+ patients in bilateral temporoparietal regions and in the hippocampus (MCI only). These temporoparietal regions had nearly complete overlap with the independently derived AD-specific metaROI regions. In a similar contrast of structural MRIs (figure 4B), patients with florbetapir− MCI had less atrophy than patients with florbetapir+ MCI in medial temporal regions including the hippocampus and amygdala, while patients with florbetapir− AD had less inferior lateral temporal atrophy than patients with florbetapir+ AD.

Figure 2 **Florbetapir standardized uptake value ratio (SUVR) distributions stratified by diagnosis and APOE4 status**



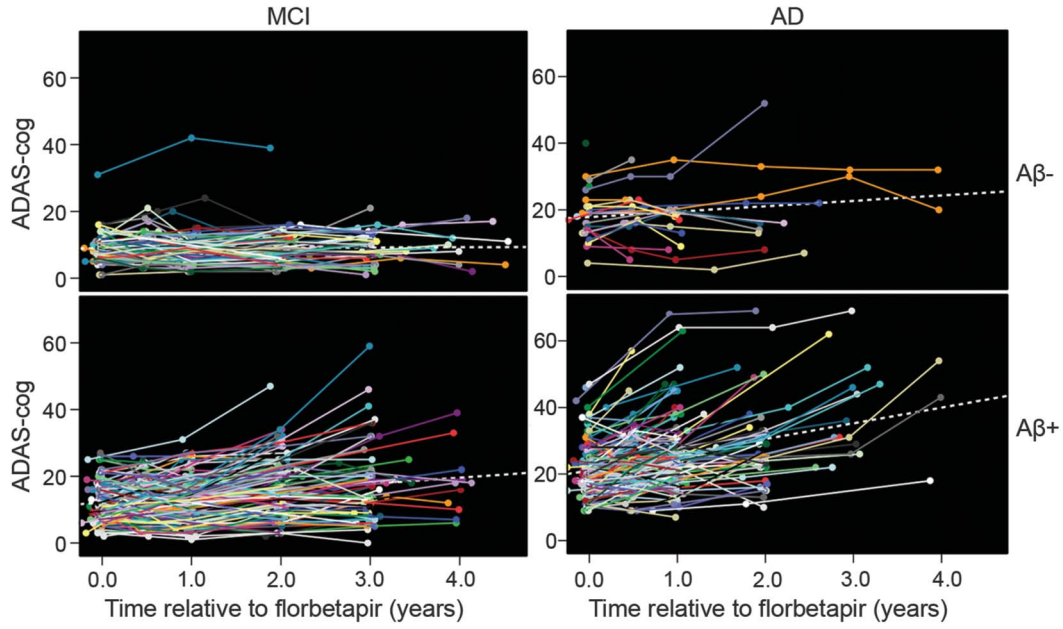
The dotted lines represent the 1.11 positivity threshold for cortical summary florbetapir SUVRs. AD = Alzheimer disease; MCI = mild cognitive impairment.

DISCUSSION A total of 15% of patients with clinically diagnosed AD and 34% of patients with amnestic MCI enrolled in ADNI were quantitatively negative on florbetapir− PET. In order to determine the likelihood that false negatives were included in this group, we first examined potential methodologic problems. CSF $A\beta_{1-42}$ measurements agreed with florbetapir in 89%–96% of cases (and about 75% of florbetapir− cases only). Potential reference region inconsistencies accounted for 0%–15% of cases. While these findings raise questions about florbetapir status of a minority of participants, we concluded that the majority of florbetapir− scans appear to accurately reflect the absence of significant fibrillar Aβ in cortex. Our data are consistent with recent work examining discrepancies in amyloid status between measurement modalities (amyloid PET, CSF Aβ, autopsy).[28,29] These discrepancies may reflect differences in Aβ binding site affinities or differing concentrations of forms of Aβ, but the majority of cases agree when more than one modality is examined.[30–33]

Patients with Aβ− MCI and AD were less "AD-like" than their Aβ+ counterparts across a number of biomarker, cognitive, and clinical variables. Patients with Aβ− AD were older. In both groups, Aβ− patients were predominantly *APOE4*−, which has been consistently observed in clinical trials[3,4] and autopsy studies.[8,9] They also declined more slowly on the ADAS-cog and MMSE, they had higher temporoparietal glucose metabolism and higher medial temporal volume, and they had lower CSF t-tau and p-tau. In other words, across an extensive set of characteristic AD biomarkers, Aβ− patients have a profile that is atypical of AD.

Clinical evaluations revealed further differences between Aβ− and Aβ+ groups. Aβ− patients were more likely to be taking medication for depression and patients with Aβ− AD had marginally higher depression scores. The latter finding is surprising because participants were screened for depression at enrollment, so our ability to detect a difference was limited by low variability in these scores. In addition, patients with Aβ− MCI were less likely to be taking medication for AD, and were less likely to convert to AD. Patients with Aβ− AD were more likely to have

Figure 3 Cognitive trajectories stratified by diagnosis and florbetapir status



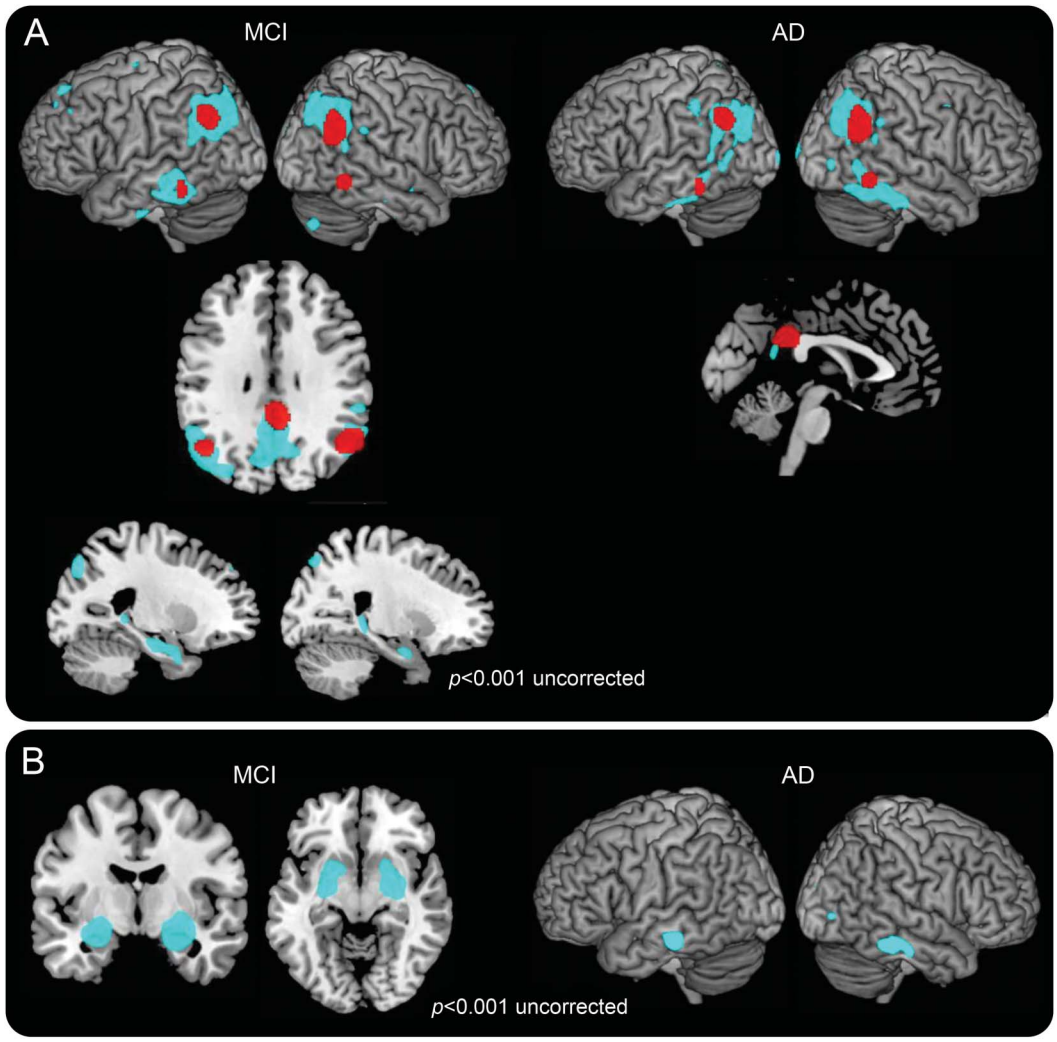
Change on the Alzheimer's Disease Assessment Scale–cognitive subscale (ADAS-cog) measured relative to the baseline florbetapir scan is shown for both florbetapir+ and florbetapir− participants within each diagnostic group. AD = Alzheimer disease; MCI = mild cognitive impairment.

a history of hypertension, and clinicians was more likely to rate their symptoms as possibly (rather than probably) due to AD. These clinical data point to the broad set of possible explanations for Aβ− patients' cognitive symptoms, as well as 2 possible specific contributors: subclinical depression and vascular abnormalities. Both depression and vascular disease have complex relationships with AD and MCI.[34–36] They are both frequently observed in the presence of AD but causal or mechanistic associations are unclear. However, our analyses yielded conflicting information, perhaps due to the nonuniformity of the Aβ− phenotype; for example, white matter hyperintensities, a marker of vascular disease, did not differ between groups. Furthermore, since the ADNI enrollment criteria excluded individuals with a history of conditions such as major depression or vascular dementia as a primary diagnosis, it is likely that the contribution of non-AD etiologies would be even greater in a community sample.

Recent autopsy studies have provided a more detailed view of the spectrum of neuropathologies observed in patients with clinically diagnosed AD. TDP-43, argyrophilic grain disease (AGD), hippocampal sclerosis, frontotemporal dementia, and vascular disease have been reported as primary neuropathologic diagnoses in patients with an antemortem clinical diagnosis of AD.[9] Some of these (TDP-43, AGD) are not possible to diagnose in vivo, and others are syndromes with symptoms that can overlap with AD. It is also likely that some individuals with a non-AD dementia syndrome may present as "AD-like" because of preexisting deficits or vulnerabilities. Furthermore, several comorbidities may be present simultaneously, making it difficult to pinpoint a cause of the cognitive symptoms. Of ADNI AD cases that have come to autopsy, there have been few non-AD primary diagnoses, but comorbidities have been observed in a majority of individuals.[10,37]

Although the pattern of results in MCI was broadly consistent with AD, there were several important differences. First, patients with Aβ− MCI had less cognitive and functional impairment at baseline than patients with Aβ+ MCI, whereas patients with AD did not differ. This is consistent with the frequent observation that MCI is a heterogeneous condition[38] and suggests that those with more severe or functional impairment were more likely to be Aβ+. In AD, functional impairment is a diagnostic criterion, which may have resulted in less functional variability, or smaller sample sizes may have resulted in lower ability to detect differences at baseline. Second, compared to patients with Aβ+ MCI, patients with Aβ− MCI had larger hippocampi and higher glucose metabolism in the hippocampus and surrounding medial temporal regions, while patients with AD did not differ on these measurements. While we cannot rule out a developmental explanation for the differing hippocampal volumes and hypometabolism, our findings suggest that non-AD etiologies involving less medial temporal pathology account for the cognitive

Figure 4 Voxelwise FDG-PET and voxel-based morphometry contrasts of florbetapir+ vs florbetapir− mild cognitive impairment (MCI) and Alzheimer disease (AD) participants



Results of whole-brain contrasts show regions with increased glucose metabolism for florbetapir− compared to florbetapir+ MCI and AD (A; blue, $p < 0.001$ uncorrected) and regions with increased gray matter volume for florbetapir− compared to florbetapir+ MCI and AD (B; blue, $p < 0.001$ uncorrected), controlling for age, sex, and education. Independently derived AD-specific FDG-PET previously validated regions of interest (red) used in our region of interest analysis are overlaid on the voxelwise FDG-PET results for visual comparison.

symptoms of patients with Aβ− MCI. The fact that patients with AD had low hippocampal volume that did not differ on the basis of amyloid status suggests that even those without amyloid had sufficient non-AD-related hippocampal pathology to account for their clinical diagnosis (e.g., hippocampal sclerosis).

These findings should be viewed in light of several limitations. First, although we divided participants into groups on the basis of amyloid status, patients with Aβ− AD phenotype make up a diverse set of individuals with a broad range of pathologies. Therefore a "relative absence of AD-like characteristics" may best summarize these patients rather than specific non-AD etiologies. Second, our ability to thoroughly evaluate Aβ− cases was limited by the data available in ADNI, which meant that there were extensive biomarker measurements available but clinical observations were relatively limited. Third, although we examined potential methodologic problems that could account for false-negative scans, there are additional culprits we could not investigate specifically, such as contamination of the cerebellum by cerebral amyloid angiopathy[39] or the presence of soluble forms of Aβ, both of which could account for some CSF+/florbetapir− cases (which were the majority of discordant CSF-florbetapir cases). Finally, because we focused on florbetapir− scans, our method was not designed to detect false-positive scans, so the full set of Aβ− patients may not have been fully captured.

A more extensive understanding of patients who present with AD symptoms but lack evidence of Aβ

pathology is important for several reasons. A discrepancy between the clinical and neuropathologic diagnoses may mislead patients and families about the anticipated course of illness. Furthermore, although there are currently no existing disease-modifying drugs available for treating AD, an incorrect diagnosis may also result in inappropriate drug treatment. Finally, inclusion of Aβ− individuals in a clinical trial of an Aβ− modifying drug therapy could result in exposure to a treatment without potential benefit and reduce the statistical power of the trial, making the observation of successful Aβ− modifying treatment less likely. Our findings indicate that screening for Aβ positivity as part of enrollment criteria in clinical trials would eliminate approximately 1 out of 7 patients with AD and 1 out of 3 patients with MCI, thereby preventing administration of Aβ− modifying treatment to patients without Aβ pathology.

AUTHOR CONTRIBUTIONS

S.M. Landau was responsible for study design, drafting and editing the manuscript, data and statistical analysis, and interpretation of results. A. Horng and A. Fero carried out image and statistical analysis and revising the manuscript. W.J. Jagust contributed to study design, interpretation of results, obtaining funding, editing the manuscript, and study supervision.

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DISCLOSURE

S. Landau has previously consulted for Genentech, Avid Radiopharmaceuticals, Inc., Janssen AI, and Biogen. A. Horng and A. Fero report no disclosures relevant to the manuscript. W. Jagust has collaborated with Avid Radiopharmaceuticals, Inc. through participation in the Alzheimer's Disease Neuroimaging Initiative. He is currently a consultant to Genentech/Banner Alzheimer Institute, Novartis, and Bioclinica. Go to Neurology.org for full disclosures.

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