# Brain structure and function as mediators of the effects of amyloid on memory

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## ABSTRACT

**Objective:** The objective of this study was to test whether effects of β-amyloid (Aβ) pathology on episodic memory were mediated by metabolism and gray matter volume in the early stages of Alzheimer disease.

**Methods:** This was a prospective cohort study. We measured baseline Aβ (using florbetapir-PET), brain function (using fluorodeoxyglucose-PET), and brain structure (using MRI). A mediation analysis was performed to test whether statistical effects of Aβ positivity on cross-sectional and longitudinal episodic memory were mediated by hypometabolism or regional gray matter volume in cognitively healthy controls (CN, n = 280) and mild cognitive impairment (MCI, n = 463).

**Results:** Lower memory scores were associated with Aβ positivity (CN, mildly; MCI, strongly), smaller gray matter volumes (CN, few regions, including hippocampus; MCI, widespread), and hypometabolism. Smaller volumes and hypometabolism mediated effects of Aβ in MCI but not in CN. The strongest individual regions mediated up to approximately 25%. A combination of brain structure and function mediated up to approximately 40%. In several regions, gray matter atrophy and hypometabolism predicted episodic memory without being associated (at $p < 0.05$) with Aβ positivity.

**Conclusions:** Changes in brain structure and function appear to be, in part, downstream events from Aβ pathology, ultimately resulting in episodic memory deficits. However, Aβ pathology is also strongly related to memory deficits through mechanisms that are not quantified by these imaging measurements, and episodic memory decline is partly caused by Alzheimer disease–like brain changes independently of Aβ pathology. ***Neurology*® 2015;84:1136–1144**

## GLOSSARY

**Aβ** = β-amyloid; **AD** = Alzheimer disease; **ADNI** = Alzheimer's Disease Neuroimaging Initiative; **AVLT** = Auditory Verbal Learning Test; **CN** = cognitively healthy control; **FDG** = fluorodeoxyglucose; **GM** = gray matter; **LM** = Logical Memory; **MCI** = mild cognitive impairment; **TDP-43** = TAR DNA-binding protein 43.

Alzheimer disease (AD)-like changes in brain structure and function may be present in the absence of biomarker evidence of β-amyloid (Aβ) pathology.[1] Therefore, it would be useful to quantify to what extent effects of Aβ on cognition are explained by brain structure and function and to quantify the strength of Aβ-independent associations between cognition and brain injury biomarkers. In this study, we tested the hypotheses that (1) Aβ, and brain structure and function were associated with episodic memory deficits, (2) effects of Aβ on episodic memory were mediated by brain structure and function, and (3) brain structure and function had Aβ-independent effects on memory. Previous studies examining the relationships among Aβ, cognition, and brain structure and function have mainly analyzed hippocampus, the ventricles, or whole brain lobes in combined or small cohorts of healthy controls (CN), patients with mild cognitive impairment

**Supplemental data at Neurology.org**

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(MCI), and patients with AD dementia.[2,3] In contrast, we explored these relationships in a large number of brain regions and analyzed large groups of CN subjects and subjects with MCI separately.

## METHODS

**Study design.** This was a prospective cohort study. Baseline examinations were performed between June 2010 and December 2013. Subjects were followed with cognitive assessment for up to 3 years. Data were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, private pharmaceutical companies, and nonprofit organizations, as a public–private partnership. The principal investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California, San Francisco. For up-to-date information, see www.adni-info.org.

**Participants.** Our study population consisted of CN subjects and subjects with MCI from ADNI-2 (table 1). Inclusion/exclusion criteria are described at: http://www.adni-info.org. Briefly, all subjects included in ADNI-2 were between the ages of 55 and 90 years, had completed at least 6 years of education, were fluent in Spanish or English, and were free of any significant neurologic disease other than AD. CN subjects had Mini-Mental State Examination score ≥24 and Clinical Dementia Rating score of 0. Subjects with MCI had Mini-Mental State Examination score ≥24, objective memory loss as shown on scores on delayed recall of the Wechsler Memory Scale Logical Memory II (>0.5 SDs below the normal mean), Clinical Dementia Rating score of 0.5, preserved activities of daily living, and absence of dementia. Baseline florbetapir-PET data were available in 340 CN subjects and 518 subjects with MCI. Of these, MRI data were available and passed quality control in 281 CN subjects and 464 subjects with MCI. Of these, fluorodeoxyglucose (FDG)-PET data were available in 280 CN subjects and 463 subjects with MCI. These subjects were included in this study.

**Cognitive tests.** We used Logical Memory (LM) delayed recall (baseline and follow-up at 1, 2, and 3 years, mean [SD] follow-up 1.68 [0.89] years) and Rey Auditory Verbal Learning Test (AVLT) delayed recall (baseline and follow-up at 0.5, 1, 2, and 3 years, mean [SD] follow-up 1.73 [0.81] years).

**Florbetapir-PET.** Baseline florbetapir data were acquired and processed as described previously.[4] Subjects were classified as Aβ-positive using a previously defined cutoff for overall cortical mean standardized uptake value ratio (>1.11).[4,5] See also e-Methods on the *Neurology*® Web site at Neurology.org.

**FDG-PET.** Baseline FDG data were acquired and processed as described previously.[4] We used data from 3 regions (averaged left and right temporal, averaged lateral parietal, and averaged posterior cingulate cortex), relative to the mean of a reference region (pons and cerebellar vermis).

**Structural MRI acquisition and image processing.** Baseline structural MRI brain scans were acquired using 3-tesla MRI scanners with a standardized protocol including T1-weighted MRI scans using a sagittal volumetric magnetization-prepared rapid-acquisition gradient echo sequence.[6] Automated cortical and subcortical volume and thickness measures were performed with FreeSurfer (http://surfer.nmr.mgh.harvard.edu/fswiki).[7] All images underwent standardized quality control. We tested all available individual 47 gray matter (GM) volumes and 6 a priori–defined combinations of region-of-interest volumes (overall, temporal, limbic, parietal, frontal, and occipital regions). Right and left hemispheres were averaged. See also e-Methods.

**Statistical analysis.** To evaluate confounding factors, we tested associations between Aβ and demographic factors (age, sex, *APOE* ε4, education) using Mann–Whitney *U* tests and $\chi^2$ tests. For the mediation analysis, the models were fitted in a logical sequence:

1. Cross-sectional relationships among Aβ, GM volume, and FDG-PET were tested using ordinary least squares regression.
2. Mediation by GM or FDG-PET of effects of Aβ on cognition was tested by linear mixed-effects models. In its original formulation, statistical mediation analysis involved testing a series of relationships between the independent variable, the dependent variable, and the mediating variable, but it has been recommended to instead bootstrap the estimate of the mediated effect.[8,9] Using this modern technique, we generated bootstrapped 95% confidence intervals for the mediation effect by calculating the difference between the coefficient for the direct effect of Aβ (*c*) and the effect when adjusting for GM volume or FDG-PET (*c'*), resulting in the mediated effect *c-c'*. We also calculated the % reduction of the effect of Aβ ($\beta_{ratio}$), and we examined whether the *p* value for the association between Aβ and cognition changed from significant to nonsignificant when adjusting for GM volume or FDG-PET. Mediation was tested for biomarkers with some ($p < 0.1$) association with the cognitive test being analyzed.
3. To test whether a combination of regional GM volumes (or FDG-PET regions) explained more of the effect of Aβ than the best individual region, we generated slopes and intercepts

**Table 1** Study demographics

| | CN | | | MCI | | |
|---|---|---|---|---|---|---|
| | All | Aβ− | Aβ+ | All | Aβ− | Aβ+ |
| No. | 280 | 194 | 86 | 463 | 210 | 253 |
| Sex, M/F (% F) | 125/155 (55) | 98/96 (50) | 27/59 (69) | 257/206 (45) | 115/95 (45) | 141/112 (44) |
| Age, y | 73.6 (6.3) | 72.9 (6.3) | 75.3 (6.0) | 71.8 (7.6) | 70.2 (7.8) | 73.1 (7.1) |
| Education, y | 16.5 (2.6) | 16.7 (2.5) | 16.0 (2.8) | 16.2 (2.7) | 16.4 (2.5) | 16.0 (2.8) |
| *APOE* ε4 (% +) | 200/77 (28) | 156/36 (19) | 44/41 (48) | 239/223 (48) | 157/52 (25) | 82/171 (68) |

Abbreviations: Aβ = β-amyloid; CN = cognitively healthy control; MCI = mild cognitive impairment.
Data on age and education are mean (SD).

for cognition (linear mixed effects) and used ridge regression to fit multiple predictors, since ridge regression provides stable estimates of coefficients of correlated predictors. We compared bootstrapped effects of Aβ when adjusted only for the most influential region (hippocampal volume and angular FDG-PET) with effects when adjusted for multiple regions. We also tested whether combining hippocampal volume and angular FDG-PET changed the mediation.

We adjusted the models for age, sex, and education to avoid bias. All tests were 2-sided, and significance was determined at $p < 0.05$. All statistics were done using R (version 3.0.1, The R Foundation for Statistical Computing). See also e-Methods. This was an observational study. The term effect was used as per statistical convention to describe associations between variables.

**Standard protocol approvals, registrations, and patient consents.** The study was approved by regional ethical standards committees at involved centers. Written informed consent was obtained from all participants.

**RESULTS** There were no associations between Aβ and age (CN, $p = 0.52$; MCI, $p = 0.59$), but there were signs of imbalance for Aβ and education (CN, $p = 0.066$; MCI, $p = 0.18$). Aβ was associated with female sex in CN ($p = 0.0045$) but not in MCI ($p = 0.91$), and with *APOE* ε4 in CN and MCI ($p < 0.0001$). Cognitive data were sparsely available at longer follow-up (LM at baseline, 1, 2, and 3 years, respectively: CN, n = 280, 214, 167, 21; MCI, n = 463, 434, 308, 95; and AVLT at baseline, 0.5, 1, 2, and 3 years, respectively: CN, n = 279, 241, 214, 167, 21; MCI, n = 463, 420, 432, 307, 93), but baseline Aβ or GM volume (tested for hippocampus) did not predict missing cognitive data.

**Associations between Aβ and brain function and structure.** Direct associations between baseline Aβ and brain function and brain structure are presented in figure 1 for key regions (figure e-1 for all regions). There were no significant associations in CN, but Aβ-positive MCI had significantly smaller volumes in several regions and lower FDG-PET in all tested regions compared with Aβ-negative MCI. The greatest effect for structure was in hippocampus and for function in the angular region.

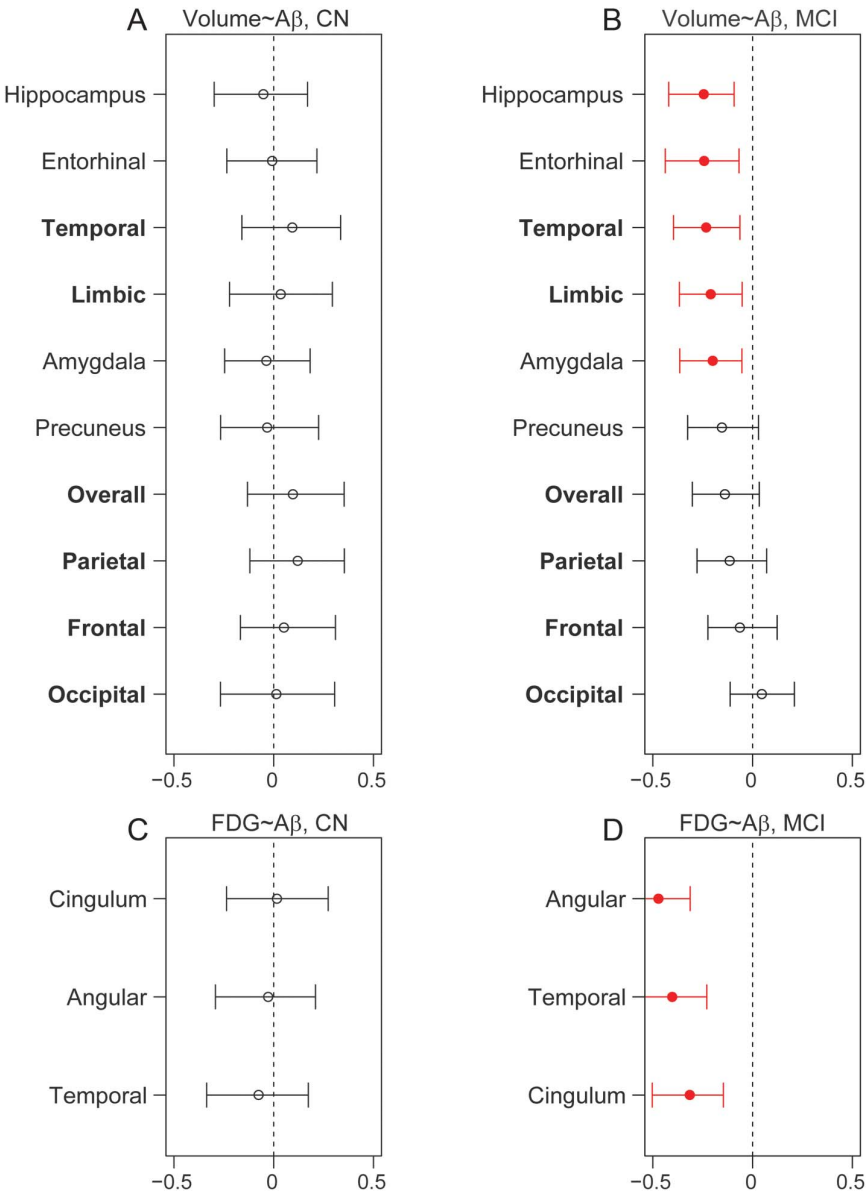
**Associations between memory and Aβ, brain function and brain structure.** Direct associations between episodic memory and Aβ, brain function and brain structure are presented in table 2 and figures e-2 and e-3. In CN, key findings included associations between low baseline LM and hypometabolism, and between accelerated decline of LM and small hippocampus and entorhinal cortex. Accelerated decline of AVLT had a borderline association with Aβ. The weak effects of Aβ in CN reduced our chances of detecting a significant mediation in the next part of the analysis. In MCI, Aβ positivity, small volumes (strong effects in, for example, hippocampus, amygdala, and entorhinal cortex), and hypometabolism were associated with lower memory scores.

**Mediation of effects of Aβ on episodic memory by brain function and structure.** There were no signs of mediation for the weak effects of Aβ on memory in CN (data not shown), but in MCI, regional volumes (primarily limbic and temporal) and FDG-PET partly mediated effects of Aβ. See figure 2 for LM and key regions (figure e-4 for all tested regions; figure e-5 for AVLT, with similar but less-pronounced effects). Hippocampus was always the most influential GM region and mediated up to 25% ($\beta_{ratio}$). FDG-PET mediated at the same order of magnitude. Whenever there was significant mediation, Aβ was still associated with lower memory scores ($p < 0.05$, in most cases $p < 0.00001$) even when adjusted for structure and function (the only exception was longitudinal AVLT, where Aβ was marginally nonsignificant when adjusting for hippocampal volume or FDG-PET; table e-1).

**Combining structure and function to explain effects of Aβ on episodic memory.** Combining multiple GM or FDG-PET regions did not change the mediation of Aβ compared with using the best individual region (data not shown). However, the combination of GM and FDG-PET accounted for a larger proportion of the effects of Aβ than the individual modalities (figure 3 and table e-1). The combination of hippocampal volume and angular FDG-PET explained 25% and 40% of the effects of Aβ on baseline and longitudinal LM, respectively (and 28% and 29% of the effects on baseline and longitudinal AVLT, respectively). For all of these except longitudinal AVLT, the combination had significantly greater mediation than the individual modalities. However, Aβ remained associated with lower memory scores even when adjusted for both structure and function ($p < 0.05$, expect for longitudinal AVLT where Aβ was no longer significant when adjusting for hippocampal volume and/or FDG-PET; table e-1).

**Effects of adjusting GM volume and FDG-PET for Aβ.** The mediation analyses were based on the assumption that abnormalities in brain structure and function were more closely related to cognitive symptoms than Aβ was. If this assumption was correct, the effects of volume or metabolism on cognition should not change considerably when models were adjusted for Aβ. This was true in all cases, with the possible exception of FDG-PET in MCI, where adjusting for Aβ reduced the effect from $\beta = 0.20$ ($p < 0.0001$) to $\beta = 0.16$ (still $p < 0.0001$, 20% change) for baseline

**Figure 1** Direct associations between Aβ and brain structure and function




β Coefficients for the effect of Aβ positivity on gray matter volumes (A, B) and FDG-PET (C, D) in CN (A, C) and MCI (B, D). The error bars are 95% confidence interval generated by nonparametric bootstrap. For regions with significant mediation (95% confidence interval excluding zero), results are shown in red. For convenience, regions are ranked by effect in MCI. Volumes, FDG-PET, and memory scores are standardized (scaled and centered). Only selected key regions were included in this plot. See figure e-1 for data on all tested regions. Aβ = β-amyloid; CN = cognitively healthy control; FDG = fluorodeoxyglucose; GM = gray matter; MCI = mild cognitive impairment.

LM, from β = 0.20 ($p < 0.0001$) to β = 0.16 (still $p < 0.0001$, 20% change) for baseline AVLT, and from β = 0.037 ($p = 0.033$) to β = 0.029 ($p = 0.099$, 22% change) for longitudinal AVLT.

**DISCUSSION** The novel findings of this study were that (1) brain structure and function mediated up to 25% of the effects of Aβ on episodic memory in MCI, (2) combining regions within imaging modalities did not increase the mediated effect, but combining GM volume with FDG-PET increased the mediated effect up to approximately 40%, and (3) small GM volumes and hypometabolism in typical AD regions were associated with episodic memory deficits without being associated with Aβ pathology, especially in CN. We also showed that (4) Aβ positivity was associated with pathologic brain structure and function in MCI, and (5)

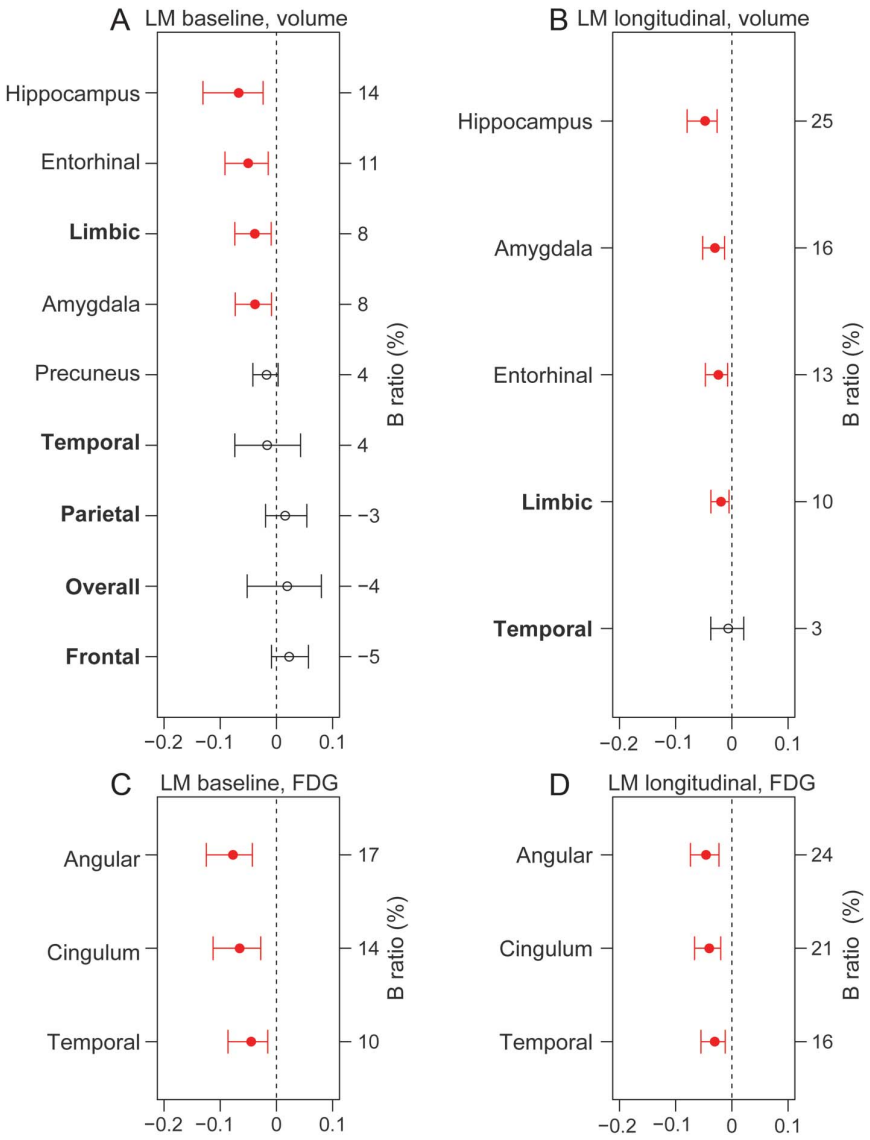
**Table 2** Direct associations between memory and Aβ pathology, GM volume, and FDG-PET

| | CN | | | | MCI | | | |
|---|---|---|---|---|---|---|---|---|
| | LM | | AVLT | | LM | | AVLT | |
| Biomarker | Baseline | Longitudinal | Baseline | Longitudinal | Baseline | Longitudinal | Baseline | Longitudinal |
| Aβ positivity | −0.096 (−0.31 to 0.11) | −0.0070 (−0.13 to 0.13) | −0.083 (−0.30 to 0.16) | −0.12 (−0.27 to 0.016) | −0.46 (−0.60 to −0.34)[a] | −0.19 (−0.27 to −0.11)[a] | −0.42 (−0.58 to −0.26)[a] | −0.078 (−0.14 to −0.0093)[a] |
| GM volume | | | | | | | | |
| Hippocampus | 0.030 (−0.079 to −0.15) | 0.15 (0.030 to 0.17)[a] | 0.026 (−0.081 to 0.14) | 0.025 (−0.056 to 0.11) | 0.30 (0.23 to 0.37)[a] | 0.13 (0.096 to 0.18)[a] | 0.34 (0.25 to 0.41)[a] | 0.047 (0.012 to 0.081)[a] |
| Amygdala | −0.0029 (−0.11 to 0.10) | 0.058 (−0.072 to 0.13) | −0.023 (−0.14 to 0.095) | 0.046 (−0.053 to 0.13) | 0.22 (0.15 to 0.28)[a] | 0.097 (0.059 to 0.14)[a] | 0.26 (0.17 to 0.33)[a] | 0.033 (0.0032 to 0.067)[a] |
| Entorhinal | −0.027 (−0.14 to 0.088) | 0.073 (0.0088 to 0.14)[a] | −0.046 (−0.16 to 0.062) | 0.034 (−0.033 to 0.094) | 0.23 (0.17 to 0.28)[a] | 0.082 (0.042 to 0.12)[a] | 0.24 (0.17 to 0.33)[a] | 0.021 (−0.012 to 0.055) |
| Overall | 0.035 (−0.082 to 0.16) | 0.032 (−0.037 to 0.11) | 0.0029 (−0.11 to 0.11) | −0.013 (−0.082 to 0.050) | 0.19 (0.11 to 0.27)[a] | 0.032 (−0.015 to 0.079) | 0.18 (0.083 to 0.27)[a] | −0.0017 (−0.039 to 0.035) |
| FDG-PET | | | | | | | | |
| Angular | 0.14 (0.025 to 0.24)[a] | 0.0052 (−0.057 to 0.069) | −0.067 (−0.18 to 0.031) | 0.065 (−0.0019 to 0.13) | 0.20 (0.14 to 0.27)[a] | 0.11 (0.076 to 0.15)[a] | 0.20 (0.13 to 0.28)[a] | 0.036 (0.0036 to 0.068)[a] |
| Temporal | 0.13 (0.036 to 0.22)[a] | 0.0079 (−0.064 to 0.077) | −0.045 (−0.16 to 0.056) | 0.050 (−0.019 to 0.11) | 0.15 (0.081 to 0.22)[a] | 0.093 (0.051 to 0.13)[a] | 0.13 (0.053 to 0.21)[a] | 0.034 (−0.0010 to 0.068) |
| Cingulate | 0.11 (0.0054 to 0.21)[a] | −0.0043 (−0.075 to 0.072) | −0.0062 (−0.10 to 0.092) | 0.044 (−0.021 to 0.11) | 0.23 (0.17 to 0.30)[a] | 0.12 (0.085 to 0.16)[a] | 0.30 (0.23 to 0.38)[a] | 0.038 (0.010 to 0.062)[a] |

Abbreviations: Aβ = β-amyloid; AVLT = Auditory Verbal Learning Test; CN = cognitively healthy control; FDG = fluorodeoxyglucose; GM = gray matter; LM = Logical Memory; MCI = mild cognitive impairment. Data are β coefficients with 95% confidence interval. β Coefficients are from linear mixed models with cognition as the dependent variable, and biomarker as the independent variable, adjusted for age, sex, and education. The main biomarker effect is used for baseline estimate and the biomarker × time interaction for longitudinal estimates. Only a selected subset of GM regions is included. See figures e-2 and e-3 for data on all tested regions.

[a] Cases where the 95% confidence interval did not include zero (indicating significant effect).

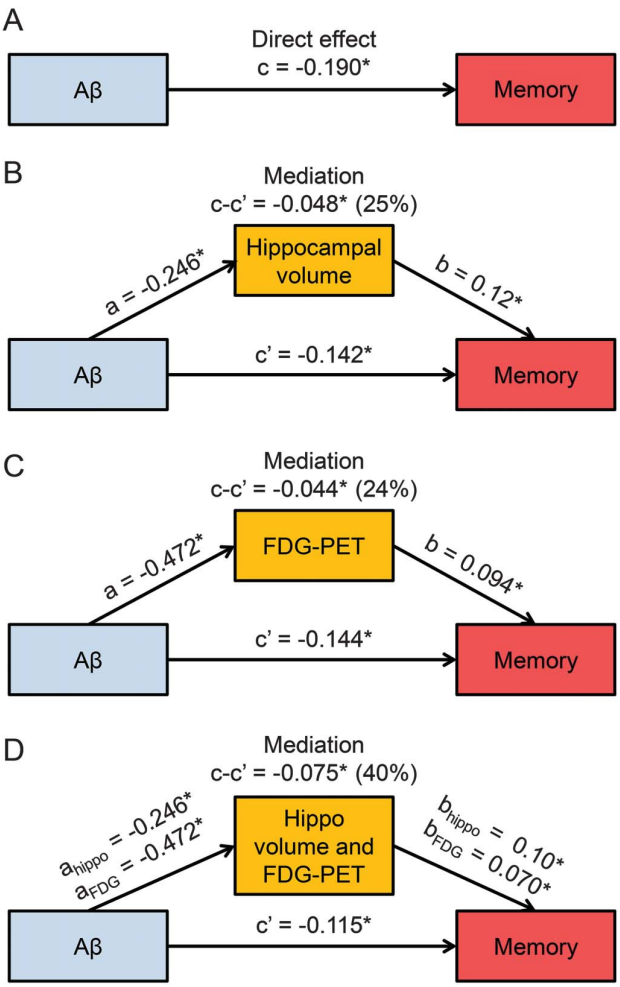
**Figure 2** Brain structure and function mediating effects of Aβ on delayed recall




Mediation by gray matter volumes (A, B) and FDG-PET (C, D) for the effect of Aβ positivity on LM delayed recall at baseline (A, C) and over time (B, D) in mild cognitive impairment. Data are indirect effects (*c*-*c*′) with 95% confidence interval. Significant mediations are shown in red. The right y-axes indicate the $\beta_{ratio}$ (the proportion of Aβ's effect that is explained by volume). For example, hippocampal atrophy explained 16% to 25% of the effect of Aβ on memory. Only selected key regions were included in this plot. See figure e-4 for data on all tested regions. Only regions with some ($p < 0.1$) direct association with the memory scores are included. Aβ = β-amyloid; FDG = fluorodeoxyglucose; LM = Logical Memory.

memory deficits were associated with small GM volumes, hypometabolism, and Aβ positivity in CN and MCI, but with differences between the groups. The results are partly in agreement with the hypothesis that Aβ pathology precedes changes in brain function and structure, ultimately resulting in loss of episodic memory. However, the results also suggest that Aβ influences episodic memory through mechanisms that are not quantified by the measurements used here, since brain structure and function only partly mediated effects, and Aβ remained associated with memory when adjusting for structure and function. Other brain changes associated with Aβ pathology may explain the additional effects on memory. This likely includes tau pathology, but could also include Lewy bodies, which are frequently seen together with Aβ pathology[10] and may accelerate cognitive decline.[11,12] The results also support that Aβ-independent changes in brain structure and function are important contributors to episodic memory deficits in the elderly,[13] since small volumes and hypometabolism

**Figure 3** Mediation of effects of Aβ on change in Logical Memory delayed recall




Path analysis showing how hippocampal volume and angular FDG-PET mediate the effect of Aβ on longitudinal change in Logical Memory delayed recall. (A) The direct effects of Aβ on memory, (B) hippocampal volume mediating effects of Aβ on memory, (C) angular FDG-PET mediating effects of Aβ on memory, and (D) the combination of hippocampal volume and FDG-PET mediating effects of Aβ on memory. The figure includes the following standardized regression coefficients: a, the effects of Aβ on hippocampal volume or FDG-PET; b, the effects of hippocampal volume or FDG-PET on memory when adjusting for Aβ; c, the direct association between Aβ and memory (without adjusting for hippocampal volume or FDG-PET); *c'*, the association between Aβ and memory when adjusting for hippocampal volume and/or FDG-PET; and *c-c'*, the mediated effect on memory (with % mediation). *$p < 0.05$. Aβ = β-amyloid; FDG = fluorodeoxyglucose; hippo = hippocampal.

were directly associated with poor memory without mediating effects of Aβ (table 2, figures 2 and e-2 to e-5).

The finding that brain structure and function mediated effects in MCI is consistent with the belief that volumetric and metabolic changes accelerate in response to Aβ pathology.[14] Few previous studies have specifically tested whether brain structure and function mediates the relationship between Aβ and memory. Our findings are in agreement with previous results that hippocampal volume was more closely related to episodic memory than Aβ deposition,[2] and that the effect of CSF $A\beta_{42}$ on episodic memory was reduced by 35% by adjusting for CSF tau and by more than 90% by adjusting for structural MRI (hippocampal volume and ventricular volume) and FDG-PET.[3] Our findings are partly in contrast with a recent study, which found that effects of Aβ on episodic memory were mediated by atrophy in frontal, parietal, and precuneus cortices in CN.[15] However, in that study, Aβ had a much stronger direct association with memory than in the CN group in our study, indicating cohort differences, as those subjects were selected to include individuals with prominent cerebrovascular disease. The relatively weak effects of Aβ in CN in this study are also different from effects of Aβ in the similar study, Australian Imaging, Biomarkers and Lifestyle,[16] which may be partly because that study included people with subjective memory concerns in the CN group, and had a higher prevalence of AD family history. Important novel contributions of the present study that sets it apart from previous studies include the separate analyses of the CN and MCI groups, the individual testing of several GM and FDG-PET regions, and the use of a very large cohort.

The finding that a combination of regions did not mediate more than the best individual region within imaging modality suggests that Aβ pathology affects episodic memory by accelerating atrophy in a group of temporal and parietal regions and hypometabolism in a temporal-parietal-cingulate network. Combining GM volume and FDG-PET mediated more than using the individual modalities alone. This is logical since cognition may be affected both by initial changes in brain function resulting from synapse dysfunction (not yet translated to gross structural changes) and by late-stage structural changes (the best neuropathologic correlate of cognitive deficits is synaptic loss[17]). These findings fit with the popular dynamic biomarker model if one allows for overlap between the dynamic phases of the trajectories of FDG-PET, structure, and cognition.[14]

The fact that small GM volumes and hypometabolism were associated with episodic memory deficits without mediating Aβ has several possible interpretations. First, the results may support the notion that AD-like clinical features and brain changes may develop partly independently of Aβ pathology. Brain changes that are associated with episodic memory deficits independently of Aβ pathology may be caused by tau, Lewy bodies, TDP-43 (TAR DNA-binding protein 43), vascular pathology, or even neurodevelopmental differences. Tau pathology is a likely candidate since it is common in the elderly population, correlates well with atrophy, and may develop partly independently of Aβ.[18] TDP-43 pathology may also contribute to Aβ-independent associations

between hippocampal volume and poor memory.[19] Subjects with Aβ-independent associations between brain structure or function and cognition may have SNAP (suspected non-Alzheimer pathophysiology[20]). Several frontal regions were associated with memory without mediating effects of Aβ in MCI (figures e-3 to e-5) and changes in these may be caused by vascular pathology or frontotemporal lobar degeneration.[21] Future studies using other imaging modalities may be useful to determine this. Other possible explanations for the Aβ-independent effects are that our Aβ biomarker may not have closely enough represented the Aβ species responsible for downstream pathology or that emerging Aβ pathology had effects on the brain before reaching overt Aβ positivity.[22]

Our findings of associations between Aβ and smaller volumes and hypometabolism in MCI but not in CN are in general agreement with previous studies, which have shown mixed results in CN with positive[23,24] and negative[25] results, but strong evidence for associations in MCI.[26] Likewise, our findings that Aβ was only mildly associated with accelerated memory decline in CN but strongly associated with memory deficits in MCI are in agreement with the literature. In CN, several studies have failed to find associations between Aβ and baseline cognition.[2,27–29] Some have reported significant effects,[30–33] but these have often been mild.[34,35] In contrast, most (but not all[36]) studies have found associations between Aβ and accelerated memory decline in CN[37,38] and both baseline and longitudinal memory deficits in MCI.[39]

Study limitations include that the use of a binary cutoff for Aβ may fail to appreciate differences in degree of Aβ pathology and the use of global Aβ hinders us from detecting subtle regional differences that may be important in early disease stages. Another limitation is that we only used cross-sectional biomarker data and longitudinal data on structure and metabolism may have increased the magnitude of the mediation.[40] Since most test were done using 95% confidence interval rather than *p* values, we did not correct for multiple comparisons. However, most significant effects were in expected regions, primarily in the temporal lobe, which makes the risk of type I errors less likely. We analyzed only LM and AVLT delayed recall, and future analyses may include a broader set of cognitive tests. We did not adjust for *APOE*, since the strong association between Aβ and *APOE* ε4 makes it difficult to parse out their individual contributions. Finally, the subjects were not consecutively recruited in clinical practice, which may reduce the generalizability of the results.

## AUTHOR CONTRIBUTIONS

Niklas Mattsson designed the study, analyzed and interpreted the data, and drafted the manuscript. Philip S. Insel designed the study, analyzed and interpreted the data, and revised the manuscript. Paul S. Aisen interpreted the data and revised the manuscript. William Jagust interpreted the data and revised the manuscript. Scott Mackin designed the study, interpreted the data, and revised the manuscript. Michael Weiner interpreted the data and revised the manuscript.

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## DISCLOSURE

N. Mattsson, P. Insel, and P. Aisen report no disclosures. W. Jagust serves as a consultant to Synarc, Inc. and Genentech. R. Mackin reports no disclosures. M. Weiner has been on scientific advisory boards for Pfizer and BOLT International; has been a consultant for Pfizer Inc., Janssen, KLJ Associates, Easton Associates, Harvard University, inThought, INC Research, Inc., University of California, Los Angeles, Alzheimer's Drug Discovery Foundation, and Sanofi-Aventis Groupe; has received funding for travel from Pfizer, AD PD meeting, Paul Sabatier University, Novartis, Tohoku University, MCI Group, France, Travel eDreams, Inc., Neuroscience School of Advanced Studies (NSAS), Danone Trading, BV, and CTAD ANT Congres; serves as an associate editor of *Alzheimer's & Dementia*; has received honoraria from Pfizer, Tohoku University, and Danone Trading, BV; has research support from Merck, Avid, DOD, and VA; and has stock options in Synarc and Elan. Go to Neurology.org for full disclosures.

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## REFERENCES

1. Knopman DS, Jack CR Jr, Wiste HJ, et al. Brain injury biomarkers are not dependent on β-amyloid in normal elderly. Ann Neurol 2013;73:472–480.
2. Mormino EC, Kluth JT, Madison CM, et al. Episodic memory loss is related to hippocampal-mediated beta-amyloid deposition in elderly subjects. Brain 2009;132:1310–1323.
3. Han SD, Gruhl J, Beckett L, et al. Beta amyloid, tau, neuroimaging, and cognition: sequence modeling of

biomarkers for Alzheimer's disease. Brain Imaging Behav 2012;6:610–620.
4. Landau SM, Mintun MA, Joshi AD, et al. Amyloid deposition, hypometabolism, and longitudinal cognitive decline. Ann Neurol 2012;72:578–586.
5. Joshi AD, Pontecorvo MJ, Clark CM, et al. Performance characteristics of amyloid PET with florbetapir F 18 in patients with Alzheimer's disease and cognitively normal subjects. J Nucl Med 2012;53:378–384.
6. Jack CR Jr, Bernstein MA, Fox NC, et al. The Alzheimer's Disease Neuroimaging Initiative (ADNI): MRI methods. J Magn Reson Imaging 2008;27:685–691.
7. Fischl B, van der Kouwe A, Destrieux C, et al. Automatically parcellating the human cerebral cortex. Cereb Cortex 2004;14:11–22.
8. MacKinnon DP, Fairchild AJ, Fritz MS. Mediation analysis. Annu Rev Psychol 2007;58:593–614.
9. Hayes AF. Beyond Baron and Kenny: statistical mediation analysis in the new millennium. Commun Monogr 2009; 76:408–420.
10. Schneider JA, Arvanitakis Z, Leurgans SE, Bennett DA. The neuropathology of probable Alzheimer disease and mild cognitive impairment. Ann Neurol 2009;66:200–208.
11. Olichney JM, Galasko D, Salmon DP, et al. Cognitive decline is faster in Lewy body variant than in Alzheimer's disease. Neurology 1998;51:351–357.
12. Mackin RS, Insel P, Zhang J, et al. Cerebrospinal fluid α-synuclein and Lewy body-like symptoms in normal controls, mild cognitive impairment, and Alzheimer's disease. J Alzheimers Dis 2015;43:1007–1016.
13. Fjell AM, McEvoy L, Holland D, Dale AM, Walhovd KB; Alzheimer's Disease Neuroimaging Initiative. Brain changes in older adults at very low risk for Alzheimer's disease. J Neurosci 2013;33:8237–8242.
14. Jack CR Jr, Knopman DS, Jagust WJ, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. Lancet Neurol 2013;12:207–216.
15. Villeneuve S, Reed BR, Wirth M, et al. Cortical thickness mediates the effect of β-amyloid on episodic memory. Neurology 2014;82:761–767.
16. Donohue MC, Sperling RA, Salmon DP, et al. The preclinical Alzheimer cognitive composite: measuring amyloid-related decline. JAMA Neurol 2014;71:961–970.
17. Terry RD, Masliah E, Salmon DP, et al. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann Neurol 1991;30:572–580.
18. Mungas D, Tractenberg R, Schneider JA, Crane PK, Bennett DA. A 2-process model for neuropathology of Alzheimer's disease. Neurobiol Aging 2014;35:301–308.
19. Josephs KA, Whitwell JL, Knopman DS, et al. Abnormal TDP-43 immunoreactivity in AD modifies clinicopathologic and radiologic phenotype. Neurology 2008;70:1850–1857.
20. Beers SR, Berger RP, Adelson PD. Neurocognitive outcome and serum biomarkers in inflicted versus noninflicted traumatic brain injury in young children. J Neurotrauma 2007;24:97–105.
21. Whitwell JL, Josephs KA. Neuroimaging in frontotemporal lobar degeneration: predicting molecular pathology. Nat Rev Neurol 2012;8:131–142.
22. Mattsson N, Insel PS, Nosheny R, et al. Emerging beta-amyloid pathology and accelerated cortical atrophy. JAMA Neurol 2014;71:725–734.
23. Becker JA, Hedden T, Carmasin J, et al. Amyloid-β associated cortical thinning in clinically normal elderly. Ann Neurol 2011;69:1032–1042.
24. Fagan AM, Head D, Shah AR, et al. Decreased cerebrospinal fluid Abeta(42) correlates with brain atrophy in cognitively normal elderly. Ann Neurol 2009;65: 176–183.
25. Wirth M, Madison CM, Rabinovici GD, Oh H, Landau SM, Jagust WJ. Alzheimer's disease neurodegenerative biomarkers are associated with decreased cognitive function but not β-amyloid in cognitively normal older individuals. J Neurosci 2013;33:5553–5563.
26. Herukka SK, Pennanen C, Soininen H, Pirttilä T. CSF Abeta42, tau and phosphorylated tau correlate with medial temporal lobe atrophy. J Alzheimers Dis 2008;14:51–57.
27. Aizenstein HJ, Nebes RD, Saxton JA, et al. Frequent amyloid deposition without significant cognitive impairment among the elderly. Arch Neurol 2008;65:1509–1517.
28. Bourgeat P, Chételat G, Villemagne VL, et al. Beta-amyloid burden in the temporal neocortex is related to hippocampal atrophy in elderly subjects without dementia. Neurology 2010;74:121–127.
29. Ossenkoppele R, van der Flier WM, Verfaillie SC, et al. Long-term effects of amyloid, hypometabolism, and atrophy on neuropsychological functions. Neurology 2014;82: 1768–1775.
30. Oh H, Madison C, Haight TJ, Markley C, Jagust WJ. Effects of age and β-amyloid on cognitive changes in normal elderly people. Neurobiol Aging 2012;33:2746–2755.
31. Rodrigue KM, Kennedy KM, Devous MD Sr, et al. β-Amyloid burden in healthy aging: regional distribution and cognitive consequences. Neurology 2012;78: 387–395.
32. Pike KE, Savage G, Villemagne VL, et al. Beta-amyloid imaging and memory in non-demented individuals: evidence for preclinical Alzheimer's disease. Brain 2007;130: 2837–2844.
33. Hedden T, Oh H, Younger AP, Patel TA. Meta-analysis of amyloid-cognition relations in cognitively normal older adults. Neurology 2013;80:1341–1348.
34. Pike KE, Ellis KA, Villemagne VL, et al. Cognition and beta-amyloid in preclinical Alzheimer's disease: data from the AIBL study. Neuropsychologia 2011;49:2384–2390.
35. Rentz DM, Locascio JJ, Becker JA, et al. Cognition, reserve, and amyloid deposition in normal aging. Ann Neurol 2010;67:353–364.
36. Driscoll I, Resnick SM, Troncoso JC, An Y, O'Brien R, Zonderman AB. Impact of Alzheimer's pathology on cognitive trajectories in nondemented elderly. Ann Neurol 2006;60:688–695.
37. Resnick SM, Sojkova J, Zhou Y, et al. Longitudinal cognitive decline is associated with fibrillar amyloid-beta measured by [11C]PiB. Neurology 2010;74:807–815.
38. Lim YY, Maruff P, Pietrzak RH, et al. Effect of amyloid on memory and non-memory decline from preclinical to clinical Alzheimer's disease. Brain 2014;137:221–231.
39. Rowe CC, Ellis KA, Rimajova M, et al. Amyloid imaging results from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging. Neurobiol Aging 2010;31: 1275–1283.
40. Ewers M, Insel P, Jagust WJ, et al. CSF biomarker and PIB-PET-derived beta-amyloid signature predicts metabolic, gray matter, and cognitive changes in nondemented subjects. Cereb Cortex 2012;22:1993–2004.