

# 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  $\beta$ -amyloid ( $A\beta$ ) 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  $A\beta$  (using florbetapir-PET), brain function (using fluorodeoxyglucose-PET), and brain structure (using MRI). A mediation analysis was performed to test whether statistical effects of  $A\beta$  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  $A\beta$  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  $A\beta$  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  $A\beta$  positivity.

**Conclusions:** Changes in brain structure and function appear to be, in part, downstream events from  $A\beta$  pathology, ultimately resulting in episodic memory deficits. However,  $A\beta$  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  $A\beta$  pathology. **Neurology® 2015;84:1136-1144**

## GLOSSARY

**$A\beta$**  =  $\beta$ -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  $\beta$ -amyloid ( $A\beta$ ) pathology.<sup>1</sup> Therefore, it would be useful to quantify to what extent effects of  $A\beta$  on cognition are explained by brain structure and function and to quantify the strength of  $A\beta$ -independent associations between cognition and brain injury biomarkers. In this study, we tested the hypotheses that (1)  $A\beta$ , and brain structure and function were associated with episodic memory deficits, (2) effects of  $A\beta$  on episodic memory were mediated by brain structure and function, and (3) brain structure and function had  $A\beta$ -independent effects on memory. Previous studies examining the relationships among  $A\beta$ , 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

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(MCI), and patients with AD dementia.<sup>2,3</sup> 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  $\geq 24$  and Clinical Dementia Rating score of 0. Subjects with MCI had Mini-Mental State Examination score  $\geq 24$ , 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.<sup>4</sup> Subjects were classified as A $\beta$ -positive using a previously defined cutoff for overall cortical

mean standardized uptake value ratio ( $>1.11$ ).<sup>4,5</sup> See also e-Methods on the *Neurology*<sup>®</sup> Web site at [Neurology.org](http://Neurology.org).

**FDG-PET.** Baseline FDG data were acquired and processed as described previously.<sup>4</sup> 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.<sup>6</sup> Automated cortical and subcortical volume and thickness measures were performed with FreeSurfer (<http://surfer.nmr.mgh.harvard.edu/fswiki>).<sup>7</sup> 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 A $\beta$  and demographic factors (age, sex, *APOE*  $\epsilon 4$ , 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 A $\beta$ , GM volume, and FDG-PET were tested using ordinary least squares regression.
2. Mediation by GM or FDG-PET of effects of A $\beta$  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.<sup>8,9</sup> 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 A $\beta$  (*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 A $\beta$  ( $\beta_{ratio}$ ), and we examined whether the *p* value for the association between A $\beta$  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 A $\beta$  than the best individual region, we generated slopes and intercepts

**Table 1** Study demographics

	CN			MCI		
	All	A $\beta$ -	A $\beta$ +	All	A $\beta$ -	A $\beta$ +
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)
<i>APOE</i> $\epsilon 4$ (% +)	200/77 (28)	156/36 (19)	44/41 (48)	239/223 (48)	157/52 (25)	82/171 (68)

Abbreviations: A $\beta$  =  $\beta$ -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 A $\beta$  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 A $\beta$  and age (CN,  $p = 0.52$ ; MCI,  $p = 0.59$ ), but there were signs of imbalance for A $\beta$  and education (CN,  $p = 0.066$ ; MCI,  $p = 0.18$ ). A $\beta$  was associated with female sex in CN ( $p = 0.0045$ ) but not in MCI ( $p = 0.91$ ), and with *APOE*  $\epsilon 4$  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 A $\beta$  or GM volume (tested for hippocampus) did not predict missing cognitive data.

**Associations between A $\beta$  and brain function and structure.** Direct associations between baseline A $\beta$  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 A $\beta$ -positive MCI had significantly smaller volumes in several regions and lower FDG-PET in all tested regions compared with A $\beta$ -negative MCI. The greatest effect for structure was in hippocampus and for function in the angular region.

**Associations between memory and A $\beta$ , brain function and brain structure.** Direct associations between episodic memory and A $\beta$ , 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 A $\beta$ . The weak effects of A $\beta$  in CN reduced our chances of detecting a significant mediation in the next part of the analysis. In MCI, A $\beta$  positivity, small volumes

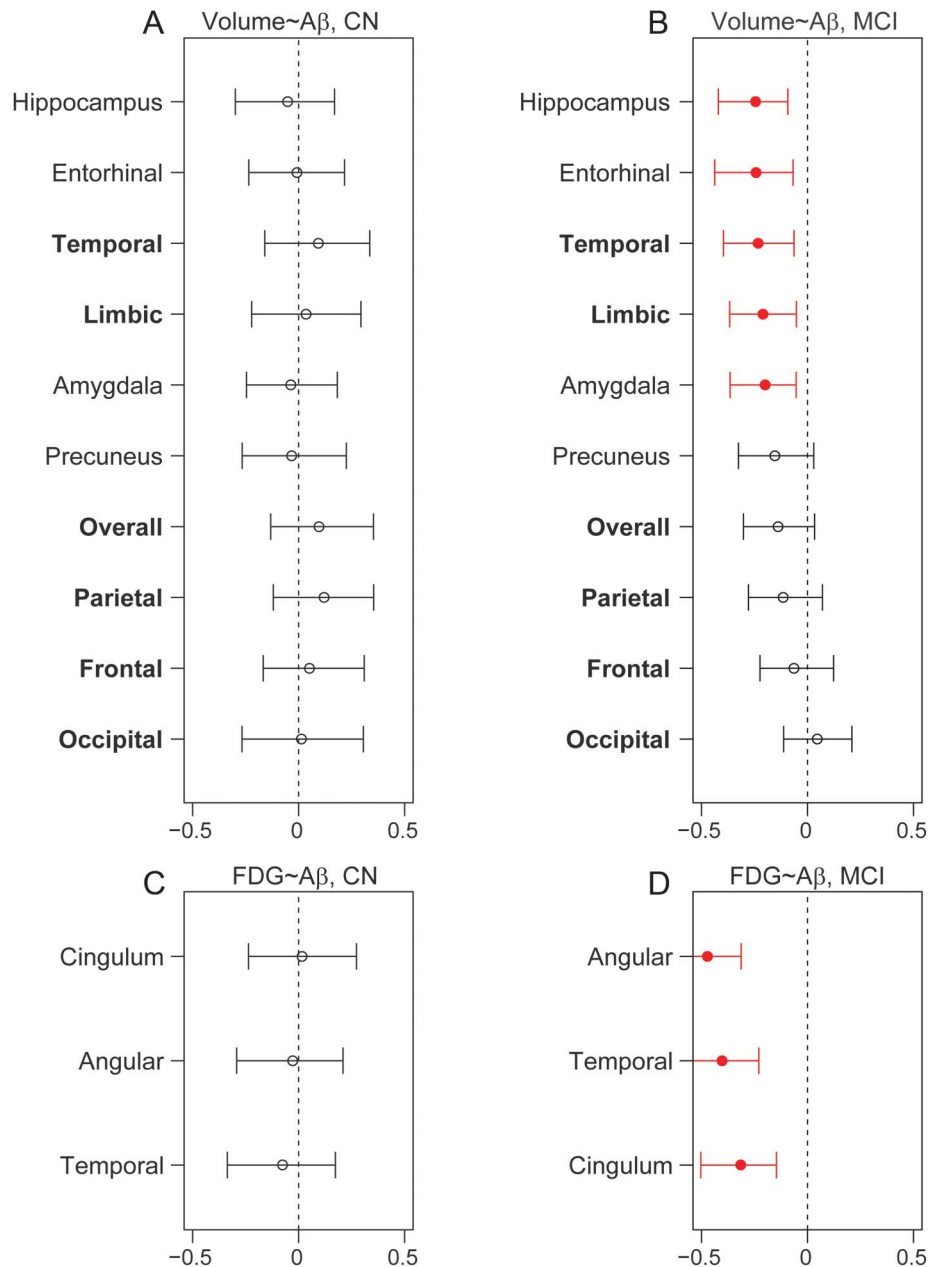
(strong effects in, for example, hippocampus, amygdala, and entorhinal cortex), and hypometabolism were associated with lower memory scores.

**Mediation of effects of A $\beta$  on episodic memory by brain function and structure.** There were no signs of mediation for the weak effects of A $\beta$  on memory in CN (data not shown), but in MCI, regional volumes (primarily limbic and temporal) and FDG-PET partly mediated effects of A $\beta$ . 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, A $\beta$  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 A $\beta$  was marginally nonsignificant when adjusting for hippocampal volume or FDG-PET; table e-1).

**Combining structure and function to explain effects of A $\beta$  on episodic memory.** Combining multiple GM or FDG-PET regions did not change the mediation of A $\beta$  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 A $\beta$  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 A $\beta$  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, A $\beta$  remained associated with lower memory scores even when adjusted for both structure and function ( $p < 0.05$ , except for longitudinal AVLT where A $\beta$  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 A $\beta$ .** The mediation analyses were based on the assumption that abnormalities in brain structure and function were more closely related to cognitive symptoms than A $\beta$  was. If this assumption was correct, the effects of volume or metabolism on cognition should not change considerably when models were adjusted for A $\beta$ . This was true in all cases, with the possible exception of FDG-PET in MCI, where adjusting for A $\beta$  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 A $\beta$  and brain structure and function



$\beta$  Coefficients for the effect of A $\beta$  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. A $\beta$  =  $\beta$ -amyloid; CN = cognitively healthy control; FDG = fluorodeoxyglucose; GM = gray matter; MCI = mild cognitive impairment.

LM, from  $\beta = 0.20$  ( $p < 0.0001$ ) to  $\beta = 0.16$  (still  $p < 0.0001$ , 20% change) for baseline AVLT, and from  $\beta = 0.037$  ( $p = 0.033$ ) to  $\beta = 0.029$  ( $p = 0.099$ , 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 A $\beta$  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 A $\beta$  pathology, especially in CN. We also showed that (4) A $\beta$  positivity was associated with pathologic brain structure and function in MCI, and (5)

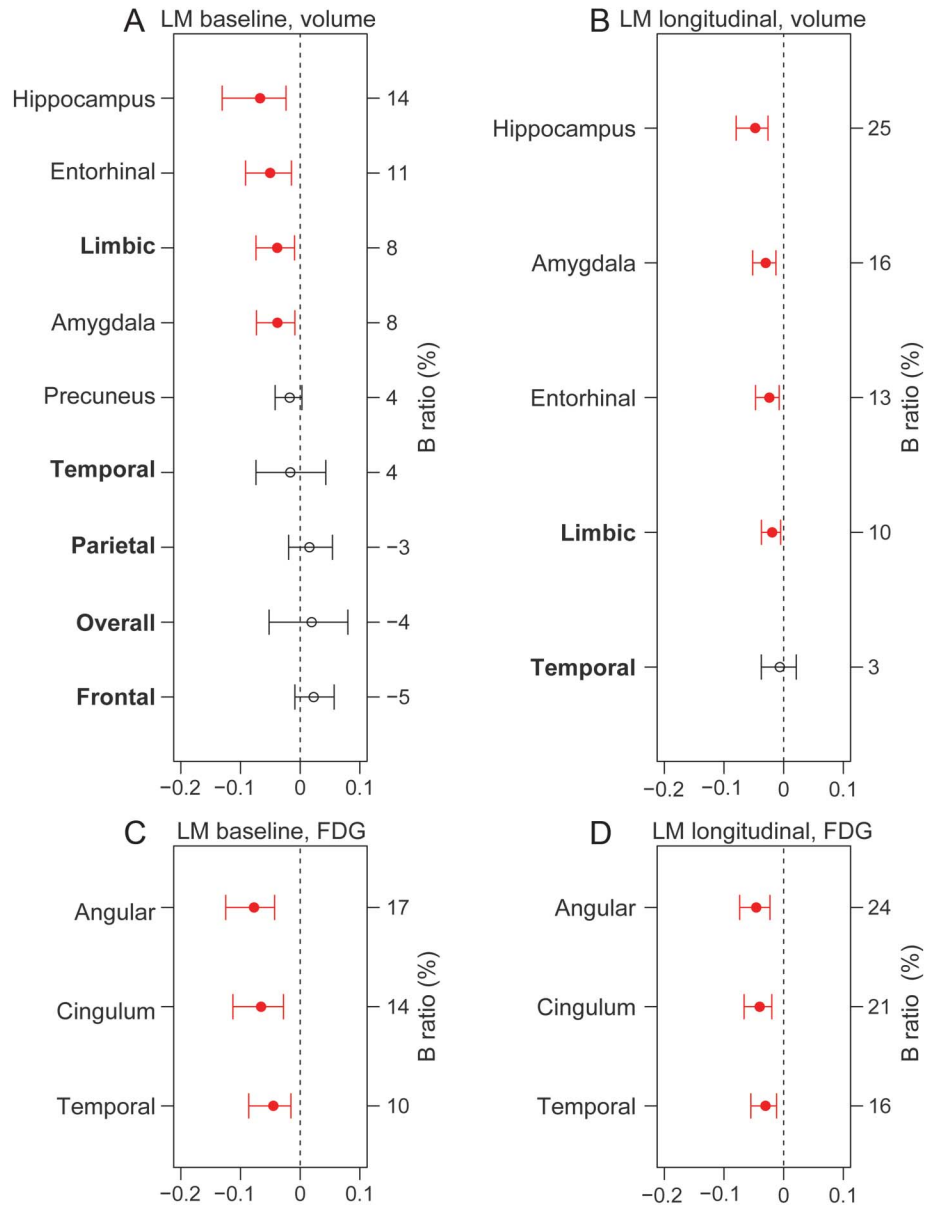
**Table 2** Direct associations between memory and A $\beta$  pathology, GM volume, and FDG-PET

Biomarker	CN				MCI			
	LM		AVLT		LM		AVLT	
	Baseline	Longitudinal	Baseline	Longitudinal	Baseline	Longitudinal	Baseline	Longitudinal
<b>A<math>\beta</math> positivity</b>	-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) <sup>a</sup>	-0.19 (-0.27 to -0.11) <sup>a</sup>	-0.42 (-0.58 to -0.26) <sup>a</sup>	-0.078 (-0.14 to -0.0093) <sup>a</sup>
<b>GM volume</b>								
<b>Hippocampus</b>	0.030 (-0.079 to -0.15)	0.15 (0.030 to 0.17) <sup>a</sup>	0.026 (-0.081 to 0.14)	0.025 (-0.056 to 0.11)	0.30 (0.23 to 0.37) <sup>a</sup>	0.13 (0.096 to 0.18) <sup>a</sup>	0.34 (0.25 to 0.41) <sup>a</sup>	0.047 (0.012 to 0.081) <sup>a</sup>
<b>Amygdala</b>	-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) <sup>a</sup>	0.097 (0.059 to 0.14) <sup>a</sup>	0.26 (0.17 to 0.33) <sup>a</sup>	0.033 (0.0032 to 0.067) <sup>a</sup>
<b>Entorhinal</b>	-0.027 (-0.14 to 0.088)	0.073 (0.0088 to 0.14) <sup>a</sup>	-0.046 (-0.16 to 0.062)	0.034 (-0.033 to 0.094)	0.23 (0.17 to 0.28) <sup>a</sup>	0.082 (0.042 to 0.12) <sup>a</sup>	0.24 (0.17 to 0.33) <sup>a</sup>	0.021 (-0.012 to 0.055)
<b>Overall</b>	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) <sup>a</sup>	0.032 (-0.015 to 0.079)	0.18 (0.083 to 0.27) <sup>a</sup>	-0.0017 (-0.039 to 0.035)
<b>FDG-PET</b>								
<b>Angular</b>	0.14 (0.025 to 0.24) <sup>a</sup>	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) <sup>a</sup>	0.11 (0.076 to 0.15) <sup>a</sup>	0.20 (0.13 to 0.28) <sup>a</sup>	0.036 (0.0036 to 0.068) <sup>a</sup>
<b>Temporal</b>	0.13 (0.036 to 0.22) <sup>a</sup>	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) <sup>a</sup>	0.093 (0.051 to 0.13) <sup>a</sup>	0.13 (0.053 to 0.21) <sup>a</sup>	0.034 (-0.0010 to 0.068)
<b>Cingulate</b>	0.11 (0.0054 to 0.21) <sup>a</sup>	-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) <sup>a</sup>	0.12 (0.085 to 0.16) <sup>a</sup>	0.30 (0.23 to 0.38) <sup>a</sup>	0.038 (0.010 to 0.062) <sup>a</sup>

Abbreviations: A $\beta$  =  $\beta$ -amyloid; AVLT = Auditory Verbal Learning Test; CN = cognitively healthy control; FDG = fluorodeoxyglucose; GM = gray matter; LM = Logical Memory; MCI = mild cognitive impairment. Data are  $\beta$  coefficients with 95% confidence interval.  $\beta$  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  $\times$  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.

<sup>a</sup>Cases where the 95% confidence interval did not include zero (indicating significant effect).

**Figure 2** Brain structure and function mediating effects of A $\beta$  on delayed recall

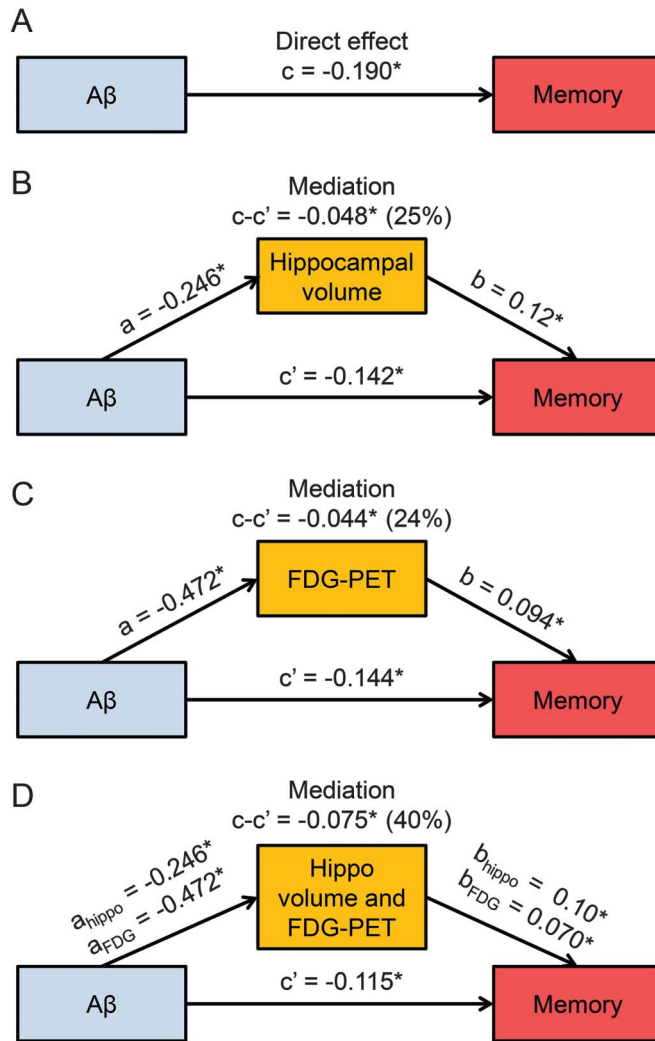


Mediation by gray matter volumes (A, B) and FDG-PET (C, D) for the effect of A $\beta$  positivity on LM delayed recall at baseline (A, C) and over time (B, D) in mild cognitive impairment. Data are indirect effects (c-c') with 95% confidence interval. Significant mediations are shown in red. The right y-axes indicate the  $\beta_{ratio}$  (the proportion of A $\beta$ 's effect that is explained by volume). For example, hippocampal atrophy explained 16% to 25% of the effect of A $\beta$  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. A $\beta$  =  $\beta$ -amyloid; FDG = fluorodeoxyglucose; LM = Logical Memory.

memory deficits were associated with small GM volumes, hypometabolism, and A $\beta$  positivity in CN and MCI, but with differences between the groups. The results are partly in agreement with the hypothesis that A $\beta$  pathology precedes changes in brain function and structure, ultimately resulting in loss of episodic memory. However, the results also suggest that A $\beta$  influences episodic memory through mechanisms that are not quantified by the measurements used here, since brain structure and function only partly mediated effects, and A $\beta$

remained associated with memory when adjusting for structure and function. Other brain changes associated with A $\beta$  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 A $\beta$  pathology<sup>10</sup> and may accelerate cognitive decline.<sup>11,12</sup> The results also support that A $\beta$ -independent changes in brain structure and function are important contributors to episodic memory deficits in the elderly,<sup>13</sup> since small volumes and hypometabolism

**Figure 3** Mediation of effects of A $\beta$  on change in Logical Memory delayed recall



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

were directly associated with poor memory without mediating effects of A $\beta$  (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 A $\beta$  pathology.<sup>14</sup> Few previous studies have specifically tested whether brain structure and function mediates the relationship between A $\beta$  and memory. Our findings are in agreement with previous results that hippocampal volume was more closely related to episodic memory than A $\beta$  deposition,<sup>2</sup>

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.<sup>3</sup> Our findings are partly in contrast with a recent study, which found that effects of A $\beta$  on episodic memory were mediated by atrophy in frontal, parietal, and precuneus cortices in CN.<sup>15</sup> However, in that study, A $\beta$  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 A $\beta$  in CN in this study are also different from effects of A $\beta$  in the similar study, Australian Imaging, Biomarkers and Lifestyle,<sup>16</sup> 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 A $\beta$  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<sup>17</sup>). 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.<sup>14</sup>

The fact that small GM volumes and hypometabolism were associated with episodic memory deficits without mediating A $\beta$  has several possible interpretations. First, the results may support the notion that AD-like clinical features and brain changes may develop partly independently of A $\beta$  pathology. Brain changes that are associated with episodic memory deficits independently of A $\beta$  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 A $\beta$ .<sup>18</sup> TDP-43 pathology may also contribute to A $\beta$ -independent associations

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

Our findings of associations between A $\beta$  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<sup>23,24</sup> and negative<sup>25</sup> results, but strong evidence for associations in MCI.<sup>26</sup> Likewise, our findings that A $\beta$  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 A $\beta$  and baseline cognition.<sup>2,27–29</sup> Some have reported significant effects,<sup>30–33</sup> but these have often been mild.<sup>34,35</sup> In contrast, most (but not all<sup>36</sup>) studies have found associations between A $\beta$  and accelerated memory decline in CN<sup>37,38</sup> and both baseline and longitudinal memory deficits in MCI.<sup>39</sup>

Study limitations include that the use of a binary cutoff for A $\beta$  may fail to appreciate differences in degree of A $\beta$  pathology and the use of global A $\beta$  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.<sup>40</sup> Since most tests 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 A $\beta$  and *APOE*  $\epsilon 4$  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

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