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Staging of amyloid β , t-tau, regional atrophy rates, and cognitive change in a nondemented cohort: Results of serial mediation analyses

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Abstract

Introduction: Current models posit a sequence of amyloid β (A β), tau, atrophy, and cognitive change leading to Alzheimer's disease, but ambiguities remain. We examined these sequences via serial mediations.

Methods: We studied normal controls, early mild cognitive impairment, and late mild cognitive impairment individuals from the Alzheimer's Disease Neuroimaging Initiative 2 database for the mediation of baseline cerebrospinal fluid A β effects on 2-year cognitive change via regional longitudinal atrophy rate (AR) alone or AR and tau.

Results: In normal controls, A β correlated directly with regional ARs and memory loss, with no mediations. In early mild cognitive impairment, tau and lateral temporal ARs serially mediated the influence of A β on memory while A β affected memory via hippocampal AR. Late mild cognitive impairment consistently showed serial mediations of tau followed by atrophy. However, A β effects on memory also continued to be specifically mediated by medial temporal ARs without intermediate tau.

Discussion: Biomarker sequences vary by region and disease state, suggesting the need to refine current cascade models.

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Keywords:

Alzheimer's disease; MCI; Amyloid β ; Tau; Cognitive aging; Longitudinal atrophy

1. Background

Recent biomarker cascade models [1–3] depict biomarker evolution as a sequence of sigmoid abnormality curves, in which amyloid β (A β) abnormality precedes abnormality in tau, which in turn leads to elevated brain

degeneration and accelerated cognitive decline (changes in cognition [Δ Cog]). The earliest model [1] featured a strict succession of abnormality curves, with A β always in the lead, but a later refinement acknowledged that preexisting tau abnormality might occur before A β while still remaining below threshold levels of detection [2]. Alternatively, a combined neurodegeneration category of tau with other markers—magnetic resonance imaging (MRI) atrophy and [18]fluorodeoxyglucose (FDG) measures of hypometabolism—might exist in levels that are barely detectable before A β abnormality [3]. Although these models acknowledge that tau may be independently deposited in brainstem, locus coeruleus, and medial temporal lobe regions (MTL), all models nonetheless make explicit predictions about

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biomarker sequences. The first is that A β is the necessary inducer of increasingly elevated tau/neurodegeneration [3]. Second, to the extent that Alzheimer's disease (AD)-related brain atrophy and FDG decline can be distinguished from effects of aging [3], these will not occur without abnormal tau. Finally, cognitive decline will not be present without abnormal neurodegeneration (see Fig. 5 in [2] and Fig. 2A–C in [3]). These predictions therefore posit a very clear ordering, in which the only possible deviation may be the presence of age-related medial temporal tau or neurodegeneration before A β abnormality.

There have been several studies investigating the sequential predictions of these models [4–9]. The main difficulty for definitive verification is that there does not exist a dataset with sufficiently long follow-up to monitor longitudinal changes, given that the buildup of brain A β is postulated to take decades [10]. In response, studies to date have relied implicitly or explicitly on the concept of mediation—the direct effects of baseline A β on cognition could be largely explained or attenuated by one or more intervening variables such as tau, MRI atrophy, or FDG—using cross-sectional or longitudinal study designs.

Mediation effects have been inferred using hierarchical models [4,5], in which variables are successively introduced one at a time to see if they diminish the effects of variables which were significant in a preceding model. Alternatively, an explicit mediation model incorporating a pathway of the form $A \rightarrow B \rightarrow C$ (see Fig. 1C in [11]) estimates whether the product of the effects $A \rightarrow B$ times $B \rightarrow C$ significantly reduces the direct unmediated effect $A \rightarrow C$. Studies using explicit modeling have investigated the roles of cortical atrophy [8], regional atrophy and FDG [7] or regional baseline and change in FDG [6] as mediators of effects of A β or tau on cognitive change.

The full sequential hypothesis of $A\beta \rightarrow \text{tau} \rightarrow \text{atrophy} \rightarrow \Delta\text{Cog}$ has been previously investigated [5]. These authors found partial support for the full sequence but also some unexpected deviations. For example, cerebrospinal fluid (CSF) A β and tau had independent effects on hippocampal baseline volume and longitudinal atrophy as well as on ventricle baseline volume and longitudinal enlargement. Meanwhile, CSF tau had an independent effect on baseline cognition. The study of the partial sequence $A\beta \rightarrow \text{tau} \rightarrow \text{atrophy}$ for hippocampus, precuneus, and (as a control) the precentral gyrus [4] also found some deviations; for example, in normals, A β directly predicted hippocampal atrophy without the mediation of tau. Meanwhile, all the explicit mediation studies [6–8] found regionally significant mediation effects of regional cortical atrophy [7,8] or FDG decline [6,7] for the effects of baseline A β or tau on cognition.

This brief survey of current literature suggests that a systematic study of the full biomarker sequence, including regional variation of atrophy rates in different diagnostic categories, may be useful to clarify the extent of applicability for the posited succession of events [1–3]. Serial mediation models—incorporating pathways of the format $A \rightarrow B_1$

$\rightarrow B_2 \rightarrow C$ and all possible subpathways (see [11], Fig. 1D)—offer the means to simultaneously test alternative mediations of the effects of A β on ΔCog via selected regional atrophy, with and without the influence of tau, and of tau, independent of regional atrophy. This allows evaluation of competing hypotheses. By comparison, previous mediation studies [6–8] did not incorporate all these factors and thus provided only partial tests of the full biomarker cascade, whereas the hierarchical model analysis [5] examined only a few regions of interest (ROIs). Our models included 2-year atrophy rates of 10 selected brain regions known to be involved in early tau deposition independent of A β [12,13] as well as of others known to be associated with the trajectory of cognitive decline in AD [7,14–17].

2. Methods

2.1. Study design

Data were obtained from the database of the Alzheimer's Disease Neuroimaging Initiative (ADNI) (adni.loni.usc.edu). The National Institute of Aging, the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, private pharmaceutical companies, and nonprofit organizations launched ADNI in 2003 as a public-private partnership. The primary goal of ADNI has been to test whether serial MRI, positron emission tomography, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early AD.

The principal investigator of ADNI is Michael Weiner, MD, VA Medical Center and University of California, San Francisco. For current information on ADNI, see www.adni-info.org.

2.2. Study participants

The study population was drawn from nondemented participants in the ADNI-2 database (Table 1). Inclusion/exclusion criteria are described at www.adni-info.org. Briefly, subjects in ADNI-2 are between the ages of 55 and 90 years at enrollment, have completed at least six years of education, and are free of any significant neurological disease other than AD. Normal controls (CNs) are distinguished from MCI categories by the Clinical Dementia Rating [18] score of 0 versus 0.5, respectively. The early mild cognitive impairment (EMCI) group differed from late mild cognitive impairment (LMCI) group only based on education-adjusted scores for the delayed paragraph recall subscore on the Wechsler Memory Scale–Revised Logical Memory II [19]; EMCI subjects were intermediate between normal subjects and LMCI.

Owing to the longitudinal aims of our analysis, we selected subjects from the ADNI-2 database having baseline CSF A β and total tau (t-tau) measurements together with baseline and 2-year cognitive measurements and structural MRI scans. Selection was made *a priori* from ADNI-2 subjects based on the availability of complete data including longitudinal imaging and measures of cognition.

Table 1
Participant characteristics

Category	CN	EMCI	LMCI	<i>P</i> value
N	80	85	64	
Age (yrs)	73.9 (6.3) ^B	71.6 (7.0)	70.6 (7.5)	.013
Gender (% male)	50	52	48	.921
Education (yrs)	17.2 (2.4) ^A	15.4 (2.6) ^B	16.7 (2.7)	<.001
APOE ε4+ (%)	25 ^{A,B}	55.2	53.1	<.001
CSF Aβ (pcg/mL)	207.4 (54.6) ^{A,B}	171.6 (54.0)	166.7 (48.2)	<.001
CSF T-tau (pcg/mL)	75.6 (41.1) ^B	85.2 (51.0)	99.2 (58.2)	.021
MEM baseline	1.14 (0.54) ^{A,B}	0.42 (0.51) ^B	0.06 (0.63)	<.001
ΔMEM (2 yrs)	0.13 (0.32) ^B	−0.02 (0.44)	−0.17 (0.48)	<.001
EF baseline	0.97 (0.74) ^{A,B}	0.43 (0.70)	0.21 (0.81)	<.001
ΔEF (2 yrs)	0.06 (0.54)	−0.04 (0.62)	−0.11 (0.67)	.253
ΔMTL (2 yrs, %)	−0.01 (0.02) ^{A,B}	−0.02 (0.02) ^B	−0.03 (0.03)	<.001
ΔLTR (2 yrs, %)	−0.01 (0.01) ^B	−0.01 (0.02) ^B	−0.02 (0.03)	<.001

Abbreviations: Aβ, amyloid β; CN, normal controls; EMCI, early mild cognitive impairment; LMCI, late mild cognitive impairment; LTR, lateral temporal lobe; MTL, medial temporal lobe; t-tau, total tau.

NOTE. Format: mean (standard deviation). Units are specified for variables where appropriate. Cognitive measures (MEM and EF) are dimensionless. ΔMTL means medial temporal lobe atrophy rate, and ΔLTR means lateral temporal lobe atrophy rate. Column five gives *P* values for group comparisons from an analysis of variance (continuous variable) or chi-square test (categorical variable). For significant overall group results, pairwise tests were performed at $\alpha = 0.05$, using Tukey-Kramer post hoc tests. A (in CN column): significantly different from EMCI; B (in CN or EMCI columns): significantly different from LMCI.

2.3. Cognitive measures

For measures of memory and executive function, we used composite scores available in the ADNI database: ADNI-MEM [20] and ADNI-EF [21], hereafter referred to as MEM and EF. Composite scores are advantageous for summarizing results of multiple tests in related domains. They afford greater precision than the individual component tests, are robust to floor and ceiling effects, and have a normal distribution with the same unit size [21] which enables direct comparisons. The MEM score accounts for version differences of its component tests [20].

2.4. CSF Aβ and T-tau

We used continuous baseline measurements of CSF Aβ and CSF t-tau. Baseline CSF t-tau and phosphorylated-tau concentrations were highly correlated ($r = 0.70$), and therefore, CSF t-tau concentrations were used for analysis. Moreover, a recent study has shown that both tau measures are significantly and linearly associated with entorhinal cortex (ERC) T807 uptake within a cognitively normal group, whereas Aβ is not [22], raising the question of the prevalence of non-amyloid-associated pathology (primary age-related tauopathy [PART] [23]) within our subjects. To further investigate this issue, we therefore examined the relationship between CSF t-tau and CSF Aβ, plotting distributions and using cluster analyses for putative clusters of low brain (high CSF) Aβ–low CSF t-tau (i.e., normal in both ranges), low brain Aβ–high t-tau (PART), and high brain Aβ–high t-tau (AD pathology).

2.5. Structural MRI acquisition and image processing

Baseline and 2-year structural MRIs were acquired on 3-Tesla scanners using the standardized ADNI protocol [24]. Voxel-wise longitudinal atrophy rates for the serial

MRI scans from each participant were computed using a tensor-based morphometry (TBM) method designed to enhance sensitivity and specificity for biological change by incorporating knowledge of likely tissue boundary locations [25,26] using an in-house processing pipeline described previously [27]. Briefly, we linearly align images at time 1 and time 2 to a “halfway space” to avoid interpolation biases when only one image is transformed [28]. Each brain scan is then corrected for field inhomogeneities using an atlas-based technique [29] and finally tissue-segmented using an algorithm sensitive to edge presence [30]. The ensuing TBM registration combines the segmented images with intensity gradients to enhance the likelihood of real edge detection and suppress noise [26]. TBM is performed in both directions, and the results are constrained to be inverses of each other [31]. The log-transformed determinant of the 3×3 Jacobian matrix of the TBM deformation at each voxel quantifies local brain change [32]. These will be referred to as log-Jacobians.

2.6. Brain atrophy rates in specified ROIs

We aimed to test mediation effects on Aβ predictions of cognitive change (either MEM or EF) by baseline t-tau and regional longitudinal atrophy. This approach was based, in part, on testing alternative mediation models of biomarker cascade hypotheses [1,3] while accounting for regional atrophy rates that may be differentially affected by CSF Aβ and t-tau concentrations because of the topographical nature of Alzheimer's pathology [15,16].

To examine regional differences in brain atrophy and their associations with CSF Aβ, CSF t-tau, and ΔCog, we selected a set of relevant ROIs *a priori* for specific analysis, which included the amygdala; entorhinal cortex; parahippocampal gyrus (PHG); inferior, middle, and superior lateral

temporal ROIs (combined and designated as lateral temporal lobe [LTR] ROI); hippocampus; thalamus; splenium; and posterior cingulum bundle. In addition, because of recent findings of association with cognitive decline, we added the insula [7] and superior longitudinal fasciculus, parieto-temporal branch [17].

The posterior cingulum bundle, splenium, thalamus, and insula were drawn in-house by an experienced anatomist and have been used in previous publications from our laboratory [27,33]. The amygdala, ERC, PHG, and LTR ROIs were derived from the Desikan-Killiany Atlas of gray matter parcellations [34]. The superior longitudinal fasciculus, parieto-temporal white matter ROI was derived from the Johns Hopkins Atlas [35]. The hippocampi were segmented in each participant's native brain by an automatic atlas-based technique [36].

We computed subject regional atrophy rates as the mean log-Jacobian values over specific ROI masks.

2.7. Statistical analysis

2.7.1. Approach

We first characterized the participants in each diagnostic group by demographics, presence of the APOE $\epsilon 4$ allele, baseline A β , t-tau, and cognitive function as well as simple estimates of 2-year change in cognition, defined as the difference between the 2-year score and the baseline score. We also summarized group 2-year atrophy rates in MTL and LTR. The diagnostic groups were compared using analysis of variance, followed by Tukey-Kramer post-hoc tests for continuous variables (all of which met the assumption of normality) and chi-square test for categorical variables.

Subsequent analyses explored the univariate interrelationships between the CSF markers, regional atrophy rates, and cognitive change measures. Mixed effects regression models using all cognitive assessments taken over a period of 2 years for each subject were used for analyses with cognitive function as the outcome. Individual slopes and intercepts were accounted for using random effects. Linear regression was used for all other outcomes. Relevant covariates of age, gender, and educational achievement were included as covariates of the univariate relationships to assess the need for inclusion in subsequent analyses. All analyses were performed separately for each diagnostic group. The final analysis phase evaluated mediations of A β \rightarrow Δ Cog.

2.7.2. Mediation analysis

2.7.2.1. Serial mediation models of biomarker cascade hypotheses

We used a four-factor, serial mediation model [11] illustrated in Fig. 1. Three mediation tests were performed simultaneously. They were the triangle pathways A β \rightarrow t-tau \rightarrow Δ Cog (β_1), A β \rightarrow ROI atrophy \rightarrow Δ Cog (β_3), and the 4-pathway A β \rightarrow t-tau \rightarrow ROI atrophy \rightarrow Δ Cog (β_2). Each edge weight in Fig. 1 was derived by linear or mixed effects of regression (depending on outcome) of an outcome

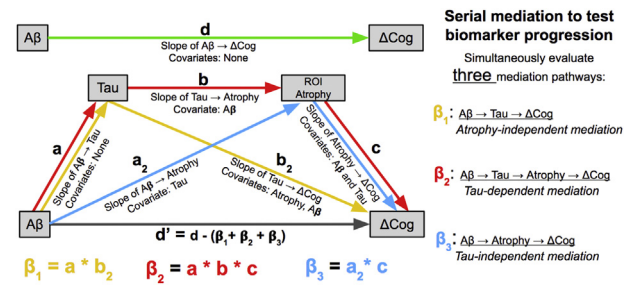


Fig. 1. Serial mediation model. Diagram of mediation model pathways showing unmediated effect of A β on cognitive change (upper diagram, green arrow) and serial mediation model including three mediation pathways (lower diagram, paths color coded). The strength of a mediation pathway (i.e., β_1 , β_2 , β_3) is the multiplicative product of the component edge weights in the pathway, as indicated at the bottom of the figure. Abbreviations: A β , amyloid β ; Δ Cog, changes in cognition.

box against one or more covariates from input boxes, in models controlling for age, gender, and education. The weights of edges in a mediation pathway were multiplied to give the effect of the mediation [11]. Thus, in Fig. 1, $\beta_1 = a*b_2$, $\beta_2 = a*b*c$, and $\beta_3 = a_2*c$.

Effect sizes for mediation pathways express the amount of change in cognition (in units of standard deviation [20,21]) per unit difference in A β (pg/mL). For atrophy regression, the effects express log-Jacobian difference (in approximate volume change percentage) per change of CSF A β .

The β_1 pathway A β \rightarrow t-tau \rightarrow Δ Cog indicates that t-tau mediates the effects of A β on cognition via some factor other than the ROI atrophy included in that model. Conversely, its nonsignificance would suggest that much of the effect of A β is mediated by pathways (with or without tau) that include the ROI in question. The β_2 pathway A β \rightarrow t-tau \rightarrow Atrophy \rightarrow Δ Cog models the serial biomarker cascade proposed by Jack et al. [3] for specific ROI atrophy rates. Thus, β_2 refines the biomarker cascade hypothesis by examining effects in local brain regions. Alternatively, the β_3 pathway A β \rightarrow Atrophy \rightarrow Δ Cog tests the mediation effect of ROI atrophy while controlling for t-tau. The effects β_2 and β_3 together provide complementary evaluations of the biomarker cascade hypothesis—comparing the full biomarker cascade with one involving atrophy but independent of t-tau—for a single selected ROI.

2.7.2.2. Computing the significance of mediation effects

Mediation pathway significance was rigorously tested using a bootstrap [37] resampling scheme of the original data. All pathways were tested simultaneously. To estimate 95% and 99% confidence intervals for mediation effects [11], we resampled the dataset with replacement 10,000 times, using the same resampling to compute all regression and mediation effects at each iteration. Analyses and computations were performed using R, version 3.2.4 [38]. If both the upper and lower bounds of a confidence interval had the same sign, then the mediation or regression effect was considered significant (i.e., not 0) at the corresponding level of confidence.

3. Results

3.1. Participant characteristics

Participant characteristics appear in Table 1 for CN (N = 80), EMCI (N = 85), and LMCI (N = 64). Subtle but significant group differences were present for age ($P = .01$) and education ($P < .0001$), with CNs being the oldest and most educated. There were striking between-group differences for prevalence of at least one APOE $\epsilon 4$ allele between the CN and MCI groups (25% in CN vs. 55% for EMCI and 53% for LMCI).

A similar group difference was found for CSF A β , with mean values above the 192 pg/mL cutoff [39] in CN group (i.e., CN mean was A β -) but below in both the MCI groups ($P < .001$). There was no significant difference in CSF A β between EMCI and LMCI groups. For CSF t-tau, CN and EMCI groups both had mean values below the cutoff of 92 pg/mL [39] (i.e., they were t-tau-), whereas the LMCI t-tau mean was above the cutoff. CN and LMCI t-tau means differed significantly ($P = .015$ for all-pairs comparison using Tukey-Kramer), but EMCI did not significantly differ from either.

A final observation is relevant for the robustness of our results. As seen in Table 1, variability of key components in the mediation pathway models—A β , t-tau, and regional atrophy—is present in all groups. A large amount of t-tau variability exists in the CN and MCI groups. Meanwhile, for representative regional atrophy rates for MTL and LTR, the standard deviations in all diagnostic groups equal or exceed the mean values.

3.2. Cognitive performance

As expected, baseline and 2-year change in MEM and EF scores all become more negative with progression from CN to EMCI and LMCI groups (Table 1).

3.3. Associations between A β , t-tau, regional atrophy, and cognitive decline

Preliminary regression analyses by cognitive group showed increasing strength of associations between baseline CSF A β and t-tau proceeding from CN ($\beta = -0.13$, $P = .22$) to EMCI ($\beta = -0.41$, $P < .001$) and LMCI ($\beta = -0.64$, $P < .001$) in models controlling for age, gender, and education.

Then, we tested for mediations. We recall that our serial model simultaneously tested three mediation pathways: $\beta 1$, with tau mediating the effects of A β on cognition, independent of regional atrophy; $\beta 2$, showing the full serial mediation of A β , tau, regional atrophy, and cognition; and $\beta 3$, with regional atrophy mediating the effects of A β without tau.

Among CNs, we found no significant mediations of A $\beta \rightarrow \Delta$ Cog. We then tested simple regressions using

the bootstrap sampling with 10,000 iterations. CSF A β correlated significantly with atrophy in several regions such as the amygdala, ERC, hippocampus, LTR, PHG, superior longitudinal fasciculus, parieto-temporal, and thalamus. The amygdala, hippocampus, and PHG (all regions within the MTL) remained significant at the 99% confidence level. A β also significantly predicted Δ MEM (which remained significant at the 99% confidence level) but not Δ EF. These relations are displayed in Tables 2 and 3.

In EMCI, we found a single significant $\beta 2$ mediation (by the sequence of t-tau and LTR regional atrophy) of A $\beta \rightarrow \Delta$ MEM and a single $\beta 3$ mediation by hippocampal atrophy without t-tau. There were no significant mediations of A $\beta \rightarrow \Delta$ EF. Mediation effects for EMCI appear in Fig. 2.

We also found four significant $\beta 1$ mediations for the models involving atrophy in the insula, posterior cingulate, splenium, and thalamus, relative to the effect of A β on MEM in EMCI (data not shown). These pathways suggest that for atrophy in those ROIs, tau mediated the effects of A β on MEM via some other means, either through atrophy in other ROIs or directly rather than through the mechanism of regional atrophy. This was the only diagnostic group that had any significant $\beta 1$ pathways.

Among LMCI, we found significant $\beta 2$ mediations of A $\beta \rightarrow \Delta$ MEM by LTR, amygdala, and PHG atrophy. The amygdala and PHG also had additional significant $\beta 3$ mediations of Δ MEM. For each of these two regions, the $\beta 3$ effect was roughly twice the magnitude of the $\beta 2$ effect. In addition, the PHG $\beta 3$ mediation remained significant at the 99% confidence level. For Δ MEM, there were also significant $\beta 3$ mediations by atrophy in ERC and hippocampus.

Table 2
Associations of A β with regional atrophy rates in CN

ROI atrophy	β (95% CI)
Amygdala	0.00011* [0.00005–0.000165]
ERC	0.000118 [0.000002–0.00022]
Hippocampus	0.000091* [0.000028–0.000152]
Insula	0.000048 [0.0–0.000094]
LTR	0.000066 [0.000015–0.000121]
PHG	0.000081* [0.000029–0.000133]
Post Cing	0.000039 [–0.000001 to 0.000085]
SLF PT	0.000053 [0.000007–0.0001]
Splenium	0.000041 [–0.000001 to 0.00192]
Thalamus	0.000057 [0.000003–0.000104]

Abbreviations: A β , amyloid β ; CI, confidence interval; CN, normal controls; ERC, entorhinal cortex; LTR, lateral temporal lobe; PHG, parahippocampal gyrus; Post Cing, posterior cingulum bundle; ROIs, regions of interest; SLF PT, superior longitudinal fasciculus, parieto-temporal.

NOTE. Regression coefficients are computed by bootstrap sampling with 10,000 iterations, controlling for age, gender, and education in CN group (N = 80). β coefficients and 95% confidence intervals are displayed. Coefficients significant at 95% confidence level are in bold. Significance at 99% confidence level is further indicated with an asterisk.

Table 3
Associations of Aβ with cognitive change and t-tau in CN

Outcome	β [95% CI]
ΔEF	0.000768 [-0.000888 to 0.002353]
ΔMEM	0.000993* [0.000295-0.001707]
CSF t-tau	-0.134486 [-0.314519 to 0.033084]

Abbreviations: CI, confidence interval; t-tau, total tau.

NOTE. Regression coefficients are computed by bootstrap sampling with 10,000 iterations, controlling for age, gender, and education in CN group (N = 80). β coefficients and 95% confidence intervals are displayed. Coefficients significant at 95% confidence level are in bold. Significance at 99% confidence level is further indicated with an asterisk.

In LMCI, there were also mediations of Aβ → ΔEF. There were significant β2 effects for splenium and thalamus atrophy, and these remained significant at the 99% confidence level. Meanwhile, PHG was involved in both β2 and β3 mediations, with β3 magnitude roughly twice as large. Mediation effects for LMCI appear in Fig. 3.



β2	95% CI	ROI Atrophy	β3	95% CI
0.000053	[-0.000044,0.000193]	Amygdala	0.000242	[-0.00005,0.00065]
0.000058	[-0.000049,0.000224]	ERC	0.000111	[-0.000081,0.000423]
0.000043	[-0.000078,0.00018]	Hippocampus	0.000293	[0.000015,0.000718]
0.000003	[-0.000045,0.000054]	Insula	-0.000009	[-0.00018,0.000423]
0.000143	[0.000006,0.000366]	LTR	0.000154	[-0.000203,0.000565]
0.000075	[-0.000013,0.00023]	PHG	0.000211	[-0.000012,0.000571]
0.000001	[-0.000063,0.000065]	Post Cing	0.000024	[-0.000112,0.000232]
0.000042	[-0.000054,0.000171]	SLF_PT	0.000101	[-0.000136,0.000441]
0.000003	[-0.000061,0.000076]	Splenium	0.000134	[-0.000108,0.000453]
0.0	[-0.000081,0.000086]	Thalamus	0.000118	[-0.000101,0.000493]

Fig. 2. Mediation effects of Aβ → ΔMEM in EMCI. Small diagrams above the table indicate β2 pathways (red color code) and β3 (blue). β2 and β3 effects express change in cognitive outcomes (units of standard deviation) per unit differences in CSFAβ (pg/mL). A decrease of Aβ correlates with exacerbated cognitive loss, so β2 and β3 are both positive effects. All mediation effects and significance are computed by bootstrap sampling with 10,000 iterations. Effect sizes and 95% confidence intervals are displayed for each ROI. Mediations significant at 95% confidence level are in bold and color coded by pathway. Significance at 99% confidence level is further indicated by an asterisk. Abbreviations: Aβ, amyloid β; CI, confidence interval; EMCI, early mild cognitive impairment; ERC, entorhinal cortex; LTR, lateral temporal lobe; PHG, parahippocampal gyrus; ROIs, regions of interest; SLF PT, superior longitudinal fasciculus, parieto-temporal.

Finally, [Supplementary Fig. 1](#) shows the results of scatter-plots for each diagnostic group, examining the prevalence of CSF Aβ and t-tau levels in CN, EMCI, and LMCI. We performed 3-means clustering, and in each group, we found clusters roughly representing the ranges of normal Aβ with normal t-tau as well as abnormal Aβ coincident with both normal and abnormal t-tau. However, the incidence of normal Aβ with high t-tau or PART was low to absent. We found 11 such participants in CN group (14%), but only three of these had t-tau levels greater than 20% above the cutoff. We found no PART participants in EMCI and only two (3%) in LMCI.

4. Discussion

To our knowledge, this is the first study using serial mediation to test predictions of biomarker sequence hypotheses. Novel findings include evidence that the succession of

biomarker abnormality varies both by anatomical region—distinguishing between medial temporal and neocortical locations—and by disease state (i.e., CN vs. EMCI vs. LMCI). A β has a direct effect on regional atrophy in normals, prominently and most significantly in MTL regions, and also in some neocortical and white matter ROIs and independently on Δ MEM (Tables 2 and 3). Furthermore, A β has continued direct effects via atrophy without tau in EMCI and LMCI, as represented by the significant β 3 mediations of Δ MEM by atrophy of MTL ROIs (Figs. 2 and 3). However, in EMCI, the sequence A β \rightarrow t-tau \rightarrow LTR atrophy \rightarrow Δ MEM also occurs, and in LMCI, this expands to other regions for both Δ MEM and Δ EF. These β 2 mediations are consistent with the predictions [1–3].

These results are consistent with, and extend, an earlier study that examined temporal relations of CSF A β and tau, longitudinal MRI change in hippocampal and ventricle volumes, and cognition, using hierarchical modeling [5]. The findings of early influence of A β on MTL atrophy in normals also corroborate the previous study examining CSF biomarker associations with volume change in hippocampus and precuneus [4]. The independence of tau and amyloid processes in normal individuals is supported by a recent study of the relations of CSF A β and tau with T807 brain tau uptake among a group of cognitively normal individuals [22]. The authors found significant associations between CSF tau and local ERC tau uptake as well as between CSF A β and PiB cortical SUVR, but the association of CSF A β and T807 in MTL regions was not significant [22].

Among the previous explicit mediation studies, our study is closest to the approach [6] which modeled baseline and longitudinal FDG change as mediators for effects of baseline CSF A β and tau on change in cognition. The other two studies [7,8] used cross-sectional brain measures to estimate mediation of regional cortical thickness [8] for effects of A β on memory or mediation of regional gray matter volumes and FDG [7] of A β effects on cognitive baseline and change. As in our study, they found regional variation of the strengths of mediation effects. However, none of the previous studies used serial mediation and hence did not evaluate the full sequence of biomarker predictions or the competing subsequences simultaneously.

According to recent imaging results [12,22,40] and established literature [13,23], tau deposition in MTL regions may initially be independent of A β but may propagate to neocortical regions under the influence of A β and is thus more strongly associated with it in these locations. Consistent with this picture, our findings suggest two scenarios for mediation of the effects of A β on Δ Cog. First, by the effect of A β directly on regional atrophy without t-tau (β 3 pathway; Fig. 1) in MTL regions until late MCI and later, by the effect of A β on t-tau leading to atrophy (β 2 pathway) in neocortex ROIs, starting among EMCI in LTR and expanding to posterior limbic (thalamus and splenium) among LMCI. Regions of the β 3 scenario correspond to the locations of the early Braak stages (I and

II) of neurofibrillary tangle deposition, whereas those of the β 2 scenario correspond to neurofibrillary tangle propagation in Braak stages III and above [16].

Our findings therefore suggest the following hypothesis. In MTL regions, A β is the determining factor for atrophy because it is interacting with t-tau that is already present. This favors β 3 mediation, whereas β 2 mediation is weaker or nonsignificant because tau deposition in MTL is independent of A β [12,22,40]. In terms of our model in Fig. 1, β 2 is small because the regression coefficient “ a ” of A β \rightarrow t-tau is weak, whereas β 3 is large because the coefficient “ a_2 ” is strong. Conversely, in neocortical regions, tau may be propagated there by A β . There, the presence of tau depends on A β and effectively absorbs the effect of A β on local atrophy, leading to β 2 significance. In terms of our Fig. 1, “ a ” is strong, and β 2 is large. Our findings of increasing association between A β and t-tau in EMCI and LMCI also support this hypothesis.

The low incidence of PART in CN and its virtual absence in EMCI and LMCI favor the view that this is occurring in the AD trajectory rather than from nonamyloid PART. In particular, Supplementary Fig. 1 suggests that the weak association of CSF A β and t-tau in CN is not due to PART (i.e., subjects with normal A β and high t-tau) but rather is due to high variability of t-tau among subjects with abnormal A β . That being said, there are nonetheless some individuals in the PART category among the normals who could be weakening our results, and so caution is appropriate.

Because MTL tau deposition has been shown to be independent of CSF A β in cognitively normal individuals [22] and because abnormal CSF t-tau (and hence MTL tau deposition [22]) occurs mainly in our subjects with abnormal A β , our data likely reflect the early effects of abnormal A β in the AD spectrum with and without early MTL tau. We conclude therefore that our hypothesis about A β interacting with pre-existent tau early in the AD spectrum is plausible.

The atrophy rates significant for the β 2 and β 3 mediations may therefore reflect the dual nature of A β 's influence to synergize with already-present t-tau in MTL areas while catalyzing the propagation of t-tau to neocortical areas. This is consistent with the hypothesized role of A β in late-onset AD (see [3], Fig. 2B,C) and also with recent findings based on cross-sectional analysis [40] suggesting that A β interacts with hippocampal and cortical tau deposition to produce neurodegeneration and also that in the absence of A β , hippocampal tauopathy alone may not lead to neurodegeneration.

4.1. Limitations

Besides atrophy, at least two recent studies have also looked at mediation effects of FDG [6,7]. This is indeed an important topic for investigation, but in the interest of simplicity, we omitted any focus of FDG in this article. This also has some justification in that the more recent biomarker model refinements combine structural MRI atrophy and FDG under the umbrella category of neurodegeneration [2,3].

A



β_2	95% CI	ROI	β_3	95% CI
0.000271	[0.000027,0.0007]	Amygdala	0.00056	[0.000054,0.001285]
0.00013	[-0.000072,0.000471]	ERC	0.000552	[0.000068,0.001206]
0.000168	[-0.000033,0.000525]	Hippocampus	0.000503	[0.000032,0.001213]
0.000039	[-0.000242,0.000332]	Insula	0.000009	[-0.00018,0.000423]
0.000385	[0.000054,0.00084]	LTR	0.000157	[-0.000203,0.000565]
0.000268	[0.000043,0.000618]	PHG	0.000556*	[0.000141,0.001123]
0.000113	[-0.000111,0.000417]	Post Cing	0.000004	[-0.000254,0.000293]
0.000128	[-0.000068,0.000429]	SLF_PT	0.000186	[-0.000098,0.000652]
0.00006	[-0.000283,0.000398]	Splenium	0.000054	[-0.000144,0.000382]
0.000157	[-0.000117,0.000514]	Thalamus	0.000145	[-0.000137,0.000542]

B

0.000266	[-0.000088,0.000813]	Amygdala	0.000549	[-0.000183,0.001498]
0.000116	[-0.000074,0.000529]	ERC	0.000465	[-0.000186,0.001287]
0.000032	[-0.000275,0.000391]	Hippocampus	0.000113	[-0.00064,0.000953]
0.000315	[-0.000111,0.000923]	Insula	0.000094	[-0.000334,0.000546]
0.000493	[-0.000035,0.001198]	LTR	0.000203	[-0.000243,0.000801]
0.000384	[0.000061,0.000932]	PHG	0.000781	[0.000196,0.001576]
0.000455	[-0.000003,0.001227]	Post Cing	-0.000027	[-0.000852,0.000733]
0.000358	[-0.000017,0.001032]	SLF_PT	0.000507	[-0.000103,0.001481]
0.000701*	[0.000125,0.001567]	Splenium	0.000323	[-0.000451,0.001089]
0.000525*	[0.000105,0.001244]	Thalamus	0.00046	[-0.000164,0.001186]

Fig. 3. LMCI patterns of mediation. Small diagrams above the table indicate β_2 pathways (red color code) and β_3 (blue) (see Fig. 1). β_2 and β_3 effects express change in cognitive outcomes (units of standard deviation) per unit differences in CSF A β (pg/ml). A decrease of A β correlates with exacerbated cognitive loss, so β_2 and β_3 are both positive effects. All mediation effects and significance are computed by bootstrap sampling with 10,000 iterations. Effect sizes and 95%

Lack of positron emission tomography imaging of local t-tau and A β deposition is another limitation to our study. Our findings and their interpretation, therefore, require further confirmation. That noted, cross-sectional t-tau imaging data are consistent with our findings [12,22,40]. Furthermore, recent publications lend support to the hypothesis that global indices of A β and t-tau may be used to make regional inferences without imaging of local deposition. First, CSF t-tau is significantly correlated with MTL tau deposition in normals [22]. Second, cortical uptake of A β in widely separated brain regions has been shown to be nonlocally associated with increased tau deposition, especially in temporal and frontal-parietal regions [41]. Third, image-based tau staging [42] suggests that in vivo spreading of tau deposition follows Braak neurofibrillary tangle stages and that these stages depend on global measures of tau. Consequently, CSF measures of A β and t-tau may be representative of predictable brain states, in which (1) the level of CSF A β is more crucial than regional deposition and (2) the level of CSF t-tau is correlated with predictable locations of tau deposition in the Braak staging order. However, a caveat is warranted. Although CSF A β levels may develop over decades, changes in CSF t-tau may be faster, which calls for caution in using it as a measure of a static brain state over the period of observation.

A third limitation relates to the unusually low incidence of PART in our cohort. Although our results are consistent with our hypothesis that in CN, high brain amyloid is enhancing toxicity of tau which is already present for subjects in the early AD spectrum, confirmation is needed. Larger studies of cognitively normal individuals, using combined tau and amyloid positron emission tomography imaging and including populations where PART is more common, will likely clarify the impact of PART on longitudinal atrophy and cognitive measures. We must also caution that PART was not entirely absent in our CN group, and so it still could have reduced the association between A β , tau, atrophy, and cognition in this relatively small group of subjects.

A fourth concern may be that our models combined baseline measures for A β and t-tau with longitudinal values for regional atrophy and cognitive change. Because A β deposition takes place on a time scale of decades [10], while early MTL deposition of tau may be age dependent [12], mediation studies of change in these variables must await the availability of decades-long datasets. In the meantime, for reasons cited in a previous paragraph, we propose that baseline measurements of CSF A β variables are not likely to evolve much over a 2-year period and so can validly test predicted sequences of biomarker abnormality within each group studied (i.e., CN, EMCI, and LMCI). However,

changes in CSF t-tau over the lag interval between CSF measurements and our structural scans may not be negligible, and this suggests caution in the interpretation of our results.

Longitudinal atrophy and cognitive decline, in contrast to change in CSF measurements of A β and tau, are robustly measurable over a 2-year interscan interval, and changes in brain structure may be more strongly correlated with future cognitive changes than baseline measurements [43]. Thus, a considerable strength of our study is the incorporation of longitudinal regional atrophy and cognitive change in serial mediations to simultaneously test alternative pathways between A β and cognition. Furthermore, longitudinal analyses within diagnostic categories emphasize the dynamic nature of progression along the hypothesized biomarker pathways and are, by definition, less susceptible to limitations introduced by group differences into cross-sectional analyses commonly used as the basis for various sequential biomarker models.

Our study may also reflect a limitation from reduced statistical power because of smaller samples sizes necessitated by analyzing each diagnostic group separately. We acknowledge this issue but note the importance of testing for patterns in each group to capture the time (i.e., sequential degree of cognitive impairment) dimension of biomarker progression. We also underline that the cognitive groups were defined by very precise changes in diagnostic scores (Clinical Dementia Rating = 0.5 for both MCI, and in addition, reduced scores for LMCI on the Wechsler Memory Scale–Revised Logical Memory II). Our results therefore imply that the specific changes in these cognitive measures correlate with a progressive unfolding of biomarker sequences. A limitation may also result from the fact that because of ADNI exclusion criteria, this cohort may not accurately reflect a broader population with respect to white matter lesions and vascular disease. For these reasons, future studies are necessary to test our results on larger and differing samples.

Finally, because we performed separate mediation analyses for atrophy in each ROI and each cognitive domain for each cognitive group, resulting in a large number of fitted models, it would be natural to incorporate a penalty for multiple comparisons to correct for potential false indications of significance (type I errors). We did not do so, however, for two reasons. First, bootstrapping [11] does not provide actual *P* values for the effect sizes but rather the distribution of their estimated values, enabling confidence intervals that estimate the significance for each mediation. As a result, we do not have the ability to rank the significances by *P* value as is typically done in corrections based on false discovery or family-wise error rates. Second, multiple comparisons corrections may be undesirable because the regions and cognitive domains were strategically chosen to be those primarily

confidence intervals are displayed for each ROI. Mediations significant at 95% confidence level are in bold and color coded by pathway. Significance at 99% confidence level is further indicated by an asterisk. (A) The mediation of A β \rightarrow Δ MEM. (B) The mediation of A β \rightarrow Δ EF. Abbreviations: A β , amyloid β ; CI, confidence interval; ERC, entorhinal cortex; LMCI, late mild cognitive impairment; LTR, lateral temporal lobe; PHG, parahippocampal gyrus; ROIs, regions of interest; SLF PT, superior longitudinal fasciculus, parieto-temporal.

affected by AD. Although corrections may reduce type I errors, they can also increase type II errors in situations where patterns of associations exist [44]. Nonetheless, our estimation of two confidence intervals at 95% and 99% was designed to give a sense of the hierarchy of effect strengths for our associations and mediations.

4.2. Conclusion

We have found that serial mediation analyses show regional differences in the interaction of A β , t-tau, brain atrophy, and cognitive decline. These findings support the prominent biomarker cascade model in neocortical areas while suggesting that A β remains an important or even determining factor in MTL atrophy leading to loss of cognition. Future biomarker models, therefore, should take into account both temporal (disease state) and spatial (anatomical regions) dimensions.

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Search Terms: Alzheimer's disease; MCI; Amyloid β ; T-tau; Cognitive aging; longitudinal atrophy.

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Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.dadm.2018.04.001>.

RESEARCH IN CONTEXT

1. Systematic review: The literature for studies involving mediation of the effects of amyloid β (A β) on cognition was reviewed. Two earlier results demonstrated that brain structure and function mediates the effects of A β . However, no study has used serial mediation to evaluate current biomarker sequences.
2. Interpretation: Serial mediation supports biomarker models of A β \rightarrow tau \rightarrow regional atrophy \rightarrow cognitive decline in early and late mild cognitive impairment for lateral temporal regions and also in late mild cognitive impairment for other neocortical and white matter regional atrophy. However, medial temporal atrophy alone continues to mediate the effects of A β . Furthermore, A β directly correlates with regional atrophy without tau in normal participants. This pattern suggests an early and continued prominent role of A β independent of tau. It also suggests regional specificity for current biomarker models.
3. Future directions: The present study's results need to be confirmed by data with local tau imaging.

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