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# APOE ε4 does not modulate amyloid-β associated neurodegeneration in preclinical Alzheimer's disease

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## Abstract

**Background and Purpose—**Among cognitively normal older individuals, the relationship between the two hallmark proteins of Alzheimer's disease (AD), amyloid-β (Aβ) and tau, the ε4 allele of apolipoprotein E (APOE ε4), and neurodegeneration is not well understood.

**Materials and Methods—**We examined 107 cognitively healthy older adults who underwent longitudinal MR imaging and baseline lumbar puncture. Within the same linear mixed effects model, we concurrently investigated main and interactive effects between APOE ε4 genotype and CSF $A\beta_{1\text{-}42}$, CSF phospo-tau ($p\text{-}tau_{181p}$) and CSF $A\beta_{1\text{-}42}$, and APOE ε4 genotype and CSF $p\text{-}tau_{181p}$ on entorhinal cortex atrophy rate. We also examined the relationship between APOE ε4, CSF $p\text{-}tau_{181p}$, and CSF $A\beta_{1\text{-}42}$ on atrophy rate of other AD-vulnerable neuroanatomic regions.

**Results—**The full model with main and interactive effects demonstrated a significant interaction only between CSF $p\text{-}tau_{181p}$ and CSF $A\beta_{1\text{-}42}$ on entorhinal cortex atrophy rate indicating elevated atrophy over time in individuals with increased CSF $p\text{-}tau_{181p}$ and decreased CSF $A\beta_{1\text{-}42}$. APOE ε4 genotype was significantly and specifically associated with CSF $A\beta_{1\text{-}42}$. However, the

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Dr. Linda K. McEvoy's spouse is CEO of CorTechs Labs, Inc.

interaction between APOE ε4 genotype and either CSF $A\beta_{1-42}$ or CSF p-$tau_{181p}$ on entorhinal cortex atrophy rate was not significant. We found similar results in other AD-vulnerable regions.

**Conclusions—**Based upon our findings and building upon prior experimental evidence, we propose a model of the pathogenic cascade underlying preclinical AD where APOE ε4 primarily influences Alzheimer's pathology via Aβ-related mechanisms and in turn, Aβ-associated neurodegeneration occurs only in the presence of phospho-tau.

**Keywords**

preclinical AD; neurodegeneration; p-tau; amyloid-β; APOE

## INTRODUCTION

Converging biochemical, molecular and genetic evidence indicates that amyloid-β (Aβ) plays a central role in the neurodegenerative process underlying Alzheimer's disease (AD). [1] The presence of Aβ initiates loss of dendritic spines and synapses [2] and contributes to the dysfunction of neuronal networks. [3] Reports based on mouse models suggest that multiple factors influence Aβ-associated toxicity. The ε4 allele of apolipoprotein E (APOE ε4), the most important genetic risk factor for late onset AD [4], accelerates the onset of Aβ deposition into plaques [5] and decreases the transport of Aβ across the blood brain barrier. [6] Reductions in tau, another hallmark protein of AD pathology, protect against Aβ-induced neuronal dysfunction [7] while the presence of tau potentiates Aβ-associated synapotoxicity. [8]

In humans, evidence from genetic-at-risk cohorts and neuropathological findings in clinically normal older individuals suggest that the pathobiological process underlying AD begins years before the onset of cognitive deficits or dementia symptoms. [9] Biomarker studies in cognitively asymptomatic older adults have demonstrated significant relationships between structural MRI measures of brain atrophy and cerebrospinal fluid (CSF) Aβ levels [10-12] enabling identification of clinically normal individuals who may be in a pre-symptomatic or preclinical stage of AD. [13]

Recent evidence from our laboratory indicates that in clinically normal older individuals and those with mild cognitive impairment, Aβ-associated volume loss occurs only in the presence of phospho-tau. [14] However, it is unknown whether APOE ε4 and CSF phospho-tau (p-tau) concurrently modulate the effect of CSF Aβ on longitudinal brain atrophy in preclinical AD. In this study, we investigated whether concurrent interactions between decreased CSF $A\beta_{1-42}$ and APOE ε4 and between decreased CSF $A\beta_{1-42}$ and increased CSF p-$tau_{181p}$ are associated with increased brain atrophy in cognitively normal older individuals.

## METHODS

Selection of participants and analysis methods for MRI and CSF biomarkers are briefly summarized here, with details provided in Supplemental Information.

We evaluated participants who were clinically diagnosed at baseline as cognitively and clinically normal healthy controls (global Clinical Dementia Rating = 0) from the

Alzheimer's Disease Neuroimaging Initiative. A total of 115 cognitively normal older individuals had undergone longitudinal MRI scanning, CSF lumbar puncture, and APOE ε4 genotyping. Of these individuals, we restricted our analyses to those participants (n = 107) with quality-assured baseline and at least one follow-up MRI scan (6 months to 3.5 years, 10% with six month follow-up, 15% with twelve month follow-up, 34% with twenty-four month follow-up, and 41% with thirty-six month follow-up) available as of December 2011. We classified all participants based on presence ("carriers") and absence ("non-carriers") of at least one APOE ε4 allele (Table 1). Using recently proposed CSF cutoffs, [15] we also classified all participants based on high (>23 pg/ml, "positive") and low (<23 pg/ml, "negative") p-tau$_{181p}$ levels, and on low (<192 pg/ml, "positive") and high (>192 pg/ml, "negative") A$\beta_{1\text{-}42}$ levels (Table 1).

We examined a total of 417 $T_1$-weighted MRI scans. We performed quantitative surface-based analysis of all MRI scans using an automated region-of-interest labeling technique [16] and primarily focused on the entorhinal cortex, a medial temporal lobe region that is selectively affected in the earliest stages of AD. [17-20] To additionally investigate neuroanatomic regions that are involved in the later stages of the disease process [17,18] and to minimize multiple comparisons, we averaged longitudinal volume change in the temporal pole, parahippocampal gyrus, inferior temporal gyrus, banks of the superior temporal sulcus, inferior parietal lobule, amygdala, and hippocampus to create an 'AD-vulnerable' region of interest (ROI) (Figure 1). Using an automated method developed in our laboratory [21] we assessed longitudinal sub-regional change in gray matter volume (atrophy) on serial MRI scans.

We asked whether p-tau$_{181p}$ and APOE ε4 independently influence Aβ-associated neurodegeneration. To investigate this question, we examined the main and interactive effects of CSF A$\beta_{1\text{-}42}$ and APOE ε4, and CSF A$\beta_{1\text{-}42}$ and CSF p-tau$_{181p}$ on entorhinal cortex atrophy rate in a mixed effects model, co-varying for the effects of age and sex. Specifically: $\Delta v = \beta_0 \times \Delta t + \beta_1$APOE ε4_status $\times \Delta t + \beta_2$CSF_A$\beta_{1\text{-}42}$_status $\times \Delta t + \beta_3$CSF_p-tau$_{181p}$_status $\times \Delta t + \beta_4$[APOE ε4_status $\times$ CSF_A$\beta_{1\text{-}42}$_status $\times \Delta t] + \beta_5$[CSF_p-tau$_{181p}$_status $\times$ CSF_A$\beta_{1\text{-}42}$_status $\times \Delta t]$ + covariates $\times \Delta t + \varepsilon$ Here, Δv = entorhinal cortex atrophy (millimeters) and Δt = change in time from baseline MRI scan (years). Using the same linear mixed effects framework, we also investigated the main and interactive effects of CSF A$\beta_{1\text{-}42}$ and APOE ε4, and CSF A$\beta_{1\text{-}42}$ and CSF p-tau$_{181p}$ on atrophy rate in the AD-vulnerable ROI.

## RESULTS

Results from the full model with both interactive terms showed that the interaction between CSF A$\beta_{1\text{-}42}$ and CSF p-tau$_{181p}$ status on entorhinal cortex atrophy rate was significant ($\beta_5$ = −0.39, SE = 0.14, p = 0.005), indicating elevated atrophy over time in individuals with positive CSF p-tau$_{181p}$ and positive CSF A$\beta_{1\text{-}42}$ status (Figure 2a) as previously reported. [14] In contrast, the interaction between CSF A$\beta_{1\text{-}42}$ and APOE ε4 on entorhinal cortex atrophy rate was not significant ($\beta_4$ = −0.17, SE = 0.18, p = 0.35). With both interaction terms in the model, the main effects of APOE ε4, CSF A$\beta_{1\text{-}42}$ status and CSF p-tau$_{181p}$ status were not significant. Follow-up analyses demonstrated that positive CSF A$\beta_{1\text{-}42}$ status was associated

with elevated entorhinal cortex atrophy rate only among CSF p-$tau_{181p}$ positive individuals (β-coefficient = −0.32, SE = 0.11, p = 0.008). There was no association between positive CSF $A\beta_{1-42}$ status and entorhinal cortex atrophy rate among CSF p-$tau_{181p}$ negative individuals (β-coefficient = 0.10, SE = 0.08, p = 0.23) (Figure 2a). There was no association between positive CSF $A\beta_{1-42}$ status and entorhinal cortex atrophy rate either among APOE ε4 carriers (β-coefficient = −0.11, SE = 0.19, p = 0.58) or non-carriers (β-coefficient = −0.02, SE = 0.08, p = 0.76) (Figure 2b).

Similar results were obtained when examining the association of CSF proteins, and APOE ε4 status on atrophy rate in the AD-vulnerable ROI: the interaction between CSF $A\beta_{1-42}$ and CSF p-$tau_{181p}$ status on AD-vulnerable ROI atrophy rate was significant (β-coefficient = −0.34, SE = 0.11, p = 0.002) but the interaction between CSF $A\beta_{1-42}$ and APOE ε4 was not (β-coefficient = −0.15, SE = 0.14, p = 0.28). None of the main effects of APOE ε4, CSF $A\beta_{1-42}$ status and CSF p-$tau_{181p}$ were significant with both interaction terms in the model. Follow-up analyses demonstrated that positive CSF $A\beta_{1-42}$ status was associated with elevated AD-vulnerable ROI atrophy rate among CSF p-$tau_{181p}$ positive individuals (β-coefficient = −0.30, SE = 0.09, p = 0.001) but not among CSF p-$tau_{181p}$ negative individuals (β-coefficient = 0.03, SE = 0.07, p = 0.61). There was no association between positive CSF $A\beta_{1-42}$ status and atrophy rate in the AD-vulnerable ROI either among APOE ε4 carriers (β-coefficient = −0.19, SE = 0.13, p = 0.09) or non-carriers (β-coefficient = −0.06, SE = 0.07, p = 0.38).

We also examined the possibility that APOE ε4 modulates AD-associated neurodegeneration via phospho-tau related mechanisms. Using the same linear mixed effects model framework described above, we concurrently examined the main and interactive effects of APOE ε4 and CSF p-$tau_{181p}$, CSF $A\beta_{1-42}$ and APOE ε4, and CSF $A\beta_{1-42}$ and CSF p-$tau_{181p}$ on atrophy rate of the entorhinal cortex and the AD-vulnerable ROI. We did not find a significant interaction between APOE ε4 and CSF p-$tau_{181p}$ either on atrophy rate of the entorhinal cortex (β-coefficient = −0.04, SE = 0.18, p = 0.78) or the AD-vulnerable ROI (β-coefficient = 0.19, SE = 0.15, p = 0.18). Importantly, even within this ‘triple’ interaction model, the only significant effect was the interaction between CSF $A\beta_{1-42}$ and CSF p-$tau_{181p}$ on atrophy rate of the entorhinal cortex (β-coefficient = −0.38, SE = 0.15, p = 0.01) and the AD-vulnerable ROI (β-coefficient = −0.41, SE = 0.12, p = 0.001).

Finally, though our results did not demonstrate a significant interaction between APOE ε4 and CSF $A\beta_{1-42}$ on longitudinal brain atrophy among HC individuals, we examined whether the presence of APOE ε4 is associated with decreased CSF $A\beta_{1-42}$ and increased CSF p-$tau_{181p}$ using a generalized linear model, co-varying for age and sex. Specifically: Logit([CSF_$A\beta_{1-42}$_status or CSF_p-$tau_{181p}$_status]) = $\beta_0 + \beta_1$APOE ε4_status + $\beta_2$Age + $\beta_3$Sex We found a significant relationship between APOE ε4 status and positive CSF $A\beta_{1-42}$ status (β-coefficient = 0.40, SE = 0.07, p = $4.82 \times 10^{-7}$) indicating increased Aβ deposition in ε4 carriers. In contrast, there was no relationship between APOE ε4 carriers and positive CSF p-$tau_{181p}$ status (β-coefficient = 0.05, SE = 0.09, p = 0.55).

## DISCUSSION

In this study, we show that in cognitively healthy older individuals although the presence of the ε4 allele is specifically associated with Aβ deposition, APOE ε4 does not affect Aβ-associated volume loss. In contrast, we found that phospho-tau modulates Aβ-associated neurodegeneration in clinically normal individuals, as previously reported. [14] These findings, in conjunction with recent experimental observations [21-22], support a conceptual model of the pathogenic cascade underlying preclinical AD (Figure 3) where APOE ε4 primarily influences Alzheimer's pathology via Aβ-related mechanisms and in turn, Aβ-associated neurodegeneration occurs only in the presence of phospho-tau. It is important to note that this model provides a representation of the disease process that can be assessed with currently validated biomarkers, not a comprehensive framework of all pathological processes occurring in the earliest stages of AD. As such, it can be expanded to include future findings such as mechanistic details regarding the effect of genetic susceptibility loci on AD-associated neurodegeneration.

These findings provide important insights into the preclinical stage of AD. Though several studies in cognitively asymptomatic older individuals have demonstrated a significant relationship between APOE ε4 genotype, Aβ deposition and neurodegeneration [10-12, 24-26], there has been limited evaluation of the role of phospho-tau in modulating these relationships. Our findings indicate that in clinically normal older individuals, Aβ deposition by itself, either in ε4 carriers or non-carriers, is not associated with volume loss; the presence of phospho-tau represents a critical link between APOE ε4 genotype, Aβ deposition and neurodegeneration. Consistent with prior reports [27, 28] our results illustrate that the ε4 allele primarily affects AD in an indirect fashion via Aβ. In contrast, these findings do not support a role for APOE ε4 either in effecting intra-cranial phospho-tau levels or modulating AD pathology via phospho-tau related mechanisms.

From a quantitative neuroimaging perspective, our results demonstrate the feasibility of using automated MRI-based measures of longitudinal brain atrophy as an *in vivo* biomarker even at the preclinical stage of the disease process. Building upon prior neuroimaging studies in cognitive normal older adults, [10-12,24-26] these findings indicate that volume loss can be detected in older individuals testing positive for both Aβ and phospho-tau. Furthermore, the pattern of atrophy detected in this study is consistent with previous neuropathological studies demonstrating neuronal loss within the entorhinal cortex in the earliest stages of AD. [19-20] Taken together, this suggests that the regionally specific volume loss occurring in a subset of cognitively normal older adults is neuropathologically consistent with early AD.

This study has limitations. One concern is that CSF biomarkers provide an indirect assessment of amyloid and neurofibrillary pathology and may not fully reflect the pathological processes underlying Alzheimer's disease. Another limitation is that we primarily focused on the APOE ε4 genotype and CSF biomarkers of the two pathologic hallmarks of AD. Additional genetic and cellular markers may also interact with Aβ to predict neurodegeneration in cognitively normal elders. Finally, the individuals examined here may represent a group of highly selected, generally healthy older adults who are

motivated to participate in research studies. These findings therefore need to be further validated on an independent, community-based cohort of older individuals who would be more representative of the general older population.

Clinically, these results indicate that a biomarker profile evaluating both Aβ and phospho-tau may better identify those older individuals who are at an elevated risk of progressing to eventual dementia than either biomarker by itself. Consistent with prior clinical observations from our laboratory, [29] our current findings suggest that early intervention trials should take into account both the phospho-tau and Aβ status of participants since older individuals with increased CSF p-tau$_{181p}$ and decreased CSF A$\beta_{1-42}$ levels are likely to have significantly elevated rates of volume loss compared to individuals with normal CSF p-tau$_{181p}$ and decreased CSF A$\beta_{1-42}$ levels. Finally, in addition to the current emphasis on Aβ, our findings identify the need for developing novel therapies that target APOE and tau related processes. It is likely that a complex interplay between multiple genetic and molecular entities determines AD pathogenesis. [30-31] As such, targeting 'upstream' events, such as neuronal lipids and cholesterol transporters that interact with APOE, in ε4 carriers with normal AD biomarker levels as well as 'downstream' events, such as tau phosphorylation and aggregation, in older individuals with both decreased CSF A$\beta_{1-42}$ and increased CSF p-tau$_{181p}$ levels may represent additionally beneficial treatment strategies.

## CONCLUSIONS

In summary, we show that in cognitively normal older individuals, phospho-tau modulates the effect of Aβ on neurodegeneration. In contrast, although the presence of the ε4 allele is specifically associated with Aβ deposition, APOE ε4 does not influence Aβ-associated volume loss. These findings provide important insights into the pathogenic cascade underlying preclinical AD and illustrate the importance of examining both Aβ and phospho-tau in secondary prevention trials.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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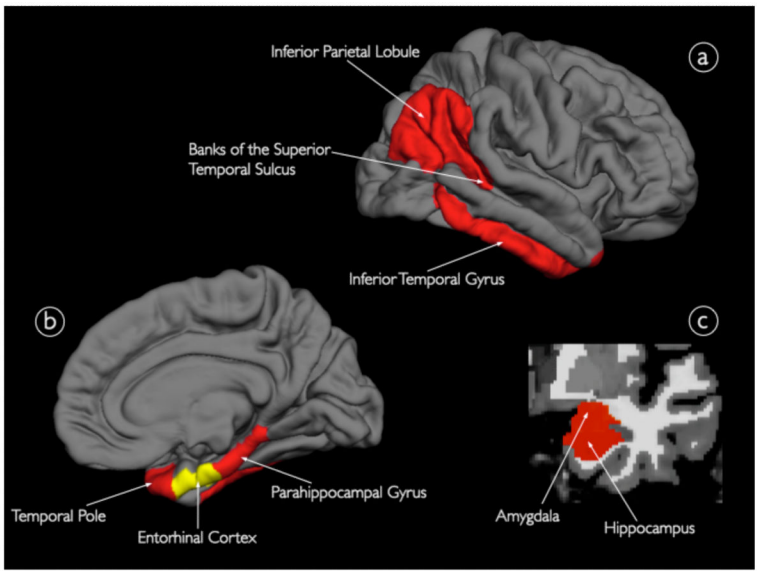


**Figure 1.**

Three-dimensional representations of the neuroanatomic regions examined in the current study (only one hemisphere is shown). All of the neocortical regions visible in the (a) lateral and (b) medial views of the gray matter surface, and (c) the two non-neocortical regions (i.e., the hippocampus and amygdala) visible in the coronal view of a T1-weighted MRI image. Regions illustrated in red constitute the 'AD-vulnerable ROI' (for further details please see manuscript text).

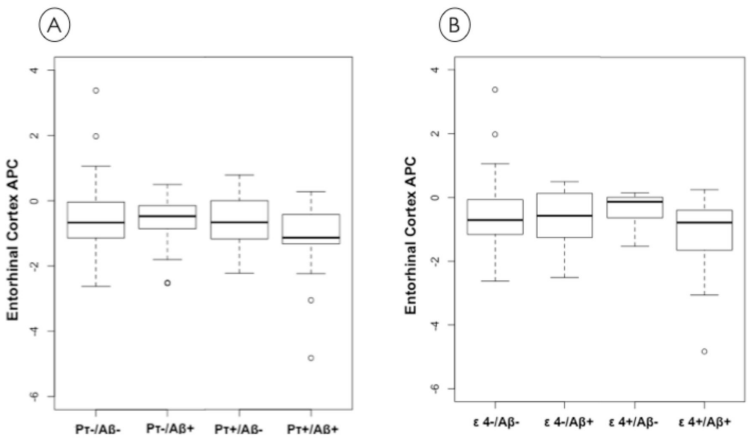


**Figure 2.**

Box and whisker plots for all HC participants illustrating entorhinal cortex atrophy rate, measured as annualized percent change (APC), based on **(a)** CSF p-tau$_{181p}$ (pτ) and CSF A$\beta_{1\text{-}42}$ (Aβ) status and **(b)** APOE ε4 (ε4) genotype and CSF A$\beta_{1\text{-}42}$ (Aβ) status. For each plot, thick black lines show the median value. Regions above and below the black line show the upper and lower quartiles, respectively. The dashed lines extend to the minimum and maximum values with outliers shown as open circles. As illustrated in (a), the pτ+/Aβ+ HC individuals demonstrated the largest cortical atrophy rate (i.e. more negative percent change). In comparison as noted in (b), the ε4+/Aβ+ HC individuals showed equivalent rates of atrophy compared to the other groups.

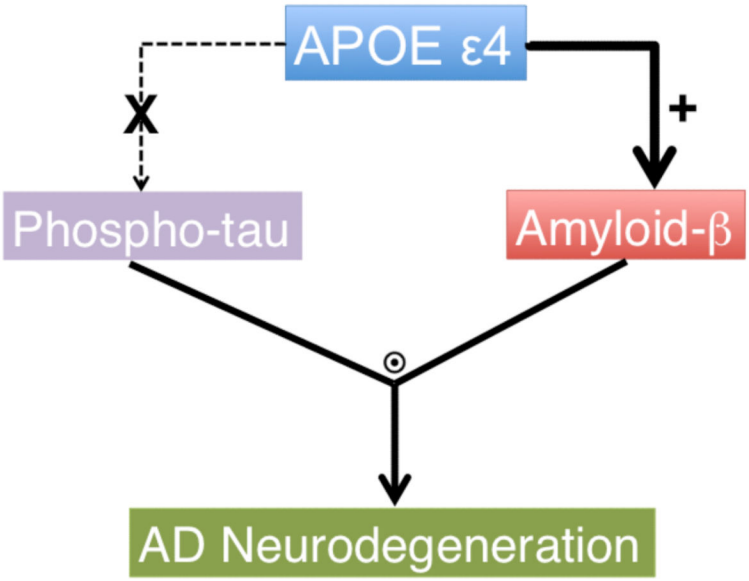


**Figure 3.**

A conceptual model of AD-associated neurodegeneration in the preclinical phase of the disease process, based on data from our mixed-effects models (please see text for details). The thickness of the arrows illustrates the magnitude of effect. ◉ illustrates an interactive effect, + illustrates a positive effect, and **X** illustrates no significant effect.

## Table 1

Demographic, clinical, and imaging data for all healthy older controls (HC) in this study, as assessed by **(a)** P-tau and Aβ status and **(b)** APOE ε4 (ε4) and Aβ status.

| | P-tau −/Aβ − (n = 46) | P-tau −/Aβ + (n = 20) | P-tau+/Aβ − (n = 19) | P-tau +/Aβ + (n = 21) | P-value |
|---|---|---|---|---|---|
| **Age, Mean (SE)** | 74.3 (0.6) | 74.9 (1.1) | 78.0 (1.4) | 78.2 (1.0) | 0.02# |
| **Female, %** | 24 | 31 | 29 | 38 | 0.59% |
| **Education Years, Mean (SE)** | 15.5 (0.4) | 14.8 (0.8) | 15.5 (0.4) | 16.7 (0.6) | 0.34# |
| **Baseline MMSE, Mean (SE)** | 29.1 (0.1) | 29.1 (0.2) | 28.8 (0.3) | 29.3 (0.2) | 0.46# |
| **Entorhinal Cortex APC, Mean (SE)** | −0.6 (0.15) | −0.6 (0.18) | −0.6 (0.18) | −1.2 (0.25) | 0.005* |
| **AD-vulnerable ROI APC, Mean** *(SE)* | −0.6 (0.08) | −0.5 (0.11) | −0.7 (0.14) | −1.1 (0.14) | 0.002* |

| | ε4 −/Aβ − (n = 61) | ε4 −/Aβ + (n = 21) | ε4 +/Aβ − (n = 5) | ε4 +/Aβ + (n = 20) | P-value |
|---|---|---|---|---|---|
| **Age, Mean (SE)** | 75.7 (0.7) | 76.2 (0.9) | 71.7 (2.5) | 77.1 (1.3) | 0.56# |
| **Female, %** | 54 | 54 | 20 | 35 | 0.23% |
| **Education Years, Mean (SE)** | 15.6 (0.3) | 15.9 (0.6) | 15 (1.1) | 15.6 (0.8) | 0.98# |
| **Baseline MMSE, Mean (SE)** | 29.1 (0.1) | 29.4 (0.2) | 28.6 (0.9) | 29 (0.2) | 0.73# |
| **Entorhinal Cortex APC, Mean (SE)** | −0.57 (0.13) | −0.67 (0.17) | −0.43 (0.30) | −1.17 (0.28) | 0.35* |
| **AD-vulnerable ROI APC, Mean (SE)** | −0.6 (0.07) | −0.78 (0.14) | −0.65 (0.23) | −1.0 (0.16) | 0.28* |

MMSE = Mini-mental status exam, APC = Annualized Percent Change, SE = Standard error of the mean, CI = Confidence Interval,

# = derived from analysis of variance,

% = derived from a chi-squared test, and

* = derived from linear mixed effects models (please see manuscript text for details).