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The Role of Vascular Endothelial Growth Factor in Neurodegeneration and Cognitive Decline: Exploring Interactions with Biomarkers of Alzheimer's Disease

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Abstract

Importance—A subset of older adults present post-mortem with Alzheimer's disease (AD) pathologic features but without any significant clinical manifestation of dementia. Vascular endothelial growth factor (VEGF) has been implicated in staving off AD-related neurodegeneration.

Objective—Evaluate whether VEGF levels are associated with brain aging outcomes (hippocampal volume, cognition). Further evaluate whether VEGF modifies relations between AD biomarkers and brain aging outcomes.

Design—Biomarker analysis using neuroimaging and neuropsychological outcomes from the Alzheimer's Disease Neuroimaging Initiative (ADNI).

Setting—Prospective longitudinal study across North America.

Participants—Participants were drawn from the ADNI and included individuals with normal cognition (n=90), mild cognitive impairment (n=130), and AD (n=59).

Main Outcome Measures—Cerebrospinal fluid (CSF) VEGF was cross-sectionally related to brain aging outcomes (hippocampal volume, episodic memory, executive function) using a general linear model and longitudinally using mixed-effects regression. AD biomarker (CSF amyloid-β42

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and total tau) x VEGF interactions evaluated the effect of VEGF on brain aging outcomes in the presence of enhanced AD biomarkers.

Results—VEGF was associated with baseline hippocampal volume (p=0.009), longitudinal hippocampal atrophy (p=0.01), and longitudinal decline in memory (p<0.0001) and executive function (p=0.003). VEGF interacted with tau in predicting longitudinal hippocampal atrophy (p<0.0001), memory decline (p=0.01), and executive function decline (p=0.0002). VEGF interacted with amyloid-β42 in predicting longitudinal memory decline (p=0.01).

Conclusions—Elevated CSF VEGF was associated with more optimal brain aging *in vivo*. The neuroprotective effect appeared strongest in the presence of enhanced AD biomarkers, suggesting that VEGF may be particularly beneficial in individuals showing early hallmarks of the AD cascade. Future work should evaluate the interaction between VEGF expression *in vitro* and pathologic burden to address potential mechanisms.

1. Introduction

Vascular endothelial growth factor (VEGF) is involved in neural development,[1] angiogenesis,[1] and blood production[1] and appears to play an essential role in the homeostasis of the adult vasculature.[2] VEGF has been investigated as a drug target for cancer,[3] but has also been implicated as a neuroprotective factor in Alzheimer's disease (AD).[4] Relative to controls, patients with AD have lower levels of serum VEGF *in vivo*[5] and lower levels of cerebral capillary VEGF expression in the superior temporal cortex, hippocampus, and brainstem.[6] In transgenic AD mice, the transplantation of stem cells overexpressing VEGF into the hippocampus reduces cognitive deficits and reverses memory defects.[7] Similarly, treating *APP* transgenic mice with VEGF results in reduced memory impairment and reduced Aβ deposition.[8] One possibility is that VEGF elevations are neuroprotective by counteracting damaging effects of the AD pathological cascade through improvements in vascular survival.[9]

VEGF has also been evaluated as a potential biomarker for AD, though results are not entirely concordant. One study evaluating intrathecal cerebrospinal fluid (CSF) levels of VEGF found that patients with AD and vascular dementia had *higher* levels than healthy controls (i.e., no neurological disease or deficit).[10] A second study found that CSF VEGF levels did not differ between AD and cognitively normal controls, further confounding the issue.[11] More recent data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) appears to be more consistent with the serum results previously reported,[5] and finds that lower levels of VEGF in CSF distinguish AD from healthy controls with 76% sensitivity and a 84% specificity.[12]

Exploration into relations between VEGF and the phenotypic presentations of AD is just beginning and may be necessary to uncover potential mechanisms of neuroprotection in elders at risk for AD. A recent study evaluated over 80 CSF analytes in relation to brain aging outcomes and found that lower levels of CSF VEGF are related to smaller hippocampi, larger ventricles, and faster decline on the Mini-Mental State Examination over 12-months.[13] Interestingly, these observations were only present in amyloid positive individuals. It is not yet clear whether an interaction between VEGF and such AD

biomarkers is specific to amyloid or whether similar interactions are also present with tau, another primary pathology in AD. More importantly, each of these outcomes (CSF biomarkers, hippocampal volume, cognitive performance) are highly correlated with diagnostic status, leaving open the possibility that the predictive power of VEGF may differ across the dementia spectrum.

The present manuscript conducts a focused, candidate analysis of CSF VEGF in relation to brain aging outcomes. First, we evaluated whether a main effect of VEGF was present cross-sectionally and longitudinally in relation to hippocampal volume and two domains of cognitive performance (episodic memory and executive function). Consistent with the theory that elevations in VEGF are neuroprotective, we hypothesized that higher VEGF levels would relate to larger hippocampal volumes and better cognitive performances. Next, we tested whether the relation between VEGF and brain aging outcomes differed between cognitive diagnostic categories. Finally, we tested the interaction between VEGF and continuous measures of CSF AD biomarkers (Aβ-42, total tau) to test whether the role of VEGF depends on the level of CSF amyloid, CSF tau, or both. Our hypothesis was that the neuroprotective effect of VEGF on brain aging outcomes (hippocampal atrophy, cognitive decline) would not be specific to one pathologic process, but would be strongest in the presence of either AD biomarker (Aβ-42, total tau).

2. Materials and Methods

Participants were drawn from the Alzheimer's Disease Neuroimaging Initiative launched in 2004 (ADNI; http://adni.loni.ucla.edu/). The original ADNI study enrolled approximately 800 participants, aged 55–90 years, excluding serious neurological disease other than AD, history of brain lesion or head trauma, and history of psychoactive medication use (for full inclusion/exclusion criteria see http://www.adni-info.org). Informed written consent was obtained from all participants at each site, and analysis of ADNI's publically available database was approved by our local Institutional Review Board prior to data analysis.

2.1 Subjects

We accessed publicly available data from ADNI on 6/01/2014. Participants were enrolled in ADNI based on criteria outlined in the ADNI protocol (http://www.adni-info.org/Scientists/AboutADNI.aspx). For the present analyses, we included all participants with CSF multiplex data that passed ADNI's quality control (QC) procedures (defined below), CSF measurement of Aβ-42 and total tau, and the neuroimaging or cognitive outcome of interest. For the neuroimaging analyses, participants had to have a FreeSurfer measure of hippocampal volume derived from 1.5T MRI data, yielding 279 participants. For the cognitive analyses, participants had to have a composite measure of memory and executive function, yielding 306 participants. Participant characteristics are presented in Table 1.

2.2 CSF Analyte and Biomarker Processing

ADNI's CSF protocol, including the quantification of Aβ-42 and total tau, has been detailed elsewhere.[14,15] For the present analyses, we compiled a dataset across the UPENN1-

UPENN5 data sources available for download and used the first measure of total tau and Aβ-42 available for each participant.

VEGF was calculated as part of a CSF multiplex proteomic processing stream using an xMAP multiplex panel (MyriadRBM; https://rbm.myriad.com/),[16] details of which are available on the ADNI website (http://adni.loni.usc.edu/wp-content/uploads/2012/01/2011Dec28-Biomarkers-Consortium-Data-Primer-FINAL1.pdf). Test-retest validation was performed across 16 participant samples to ensure reliability. Analytes were removed during ADNI's QC if the test-retest sample was <7, if the mean % difference was >35%, if the mean absolute % difference was >60%, or if the Bland Altman slope and intercept significantly differed from zero. Analytes were natural-log-transformed to better approximate a Gaussian distribution. The VEGF analyte included in this study passed each of these QC procedures.

2.3 Neuropsychological Composites

The ADNI neuropsychological protocol, including calculation of episodic memory and executive function composite measures, has been reported previously.[32,33] We leveraged a memory (ADNI-MEM) and executive function (ADNI-EF) composite score in the present analyses. ADNI-MEM included a composite z-score based on item level data from the Rey Auditory Verbal Learning Test, the AD Assessment Scale-Cognitive Test, the Mini-Mental State Examination, and Logical Memory I and II. ADNI-EF included item level data from the Trail Making Test Parts A and B, Digit Span Backward, Digit Symbol, Animal Fluency, Vegetable Fluency, and Clock Drawing Test.

2.4 Quantification of Hippocampal Volume and Hippocampal Atrophy

The ADNI neuroimaging protocol has been reported in detail elsewhere.[17] Images for the current study included original uncorrected 1.5T T1-weighted high-resolution three-dimensional structural data. Cortical reconstruction and volumetric segmentation were performed with the FreeSurfer image analysis suite version 4.3 (http://surfer.nmr.mgh.harvard.edu/).[18–20] FreeSurfer processing in ADNI has been described previously.[21] An early version of the longitudinal image processing framework was used to process the sequential scans.[22] Left hippocampal volume was the primary outcome measurement and intracranial volume (ICV) was included as a covariate in all volumetric analyses; both of which were defined by FreeSurfer.[23]

2.5 Statistical Analyses

All statistical analyses were performed in R (version 2.15.2; http://www.r-project.org/). Covariates included age, sex, education, cognitive diagnosis, and ICV (for neuroimaging analyses). Significance was set a priori as α=0.05.

2.5.1 Statistical Analyses: VEGF Main Effects on Brain Aging—Baseline effects were estimated using a general linear model for each of the three outcomes (left hippocampal volume, ADNI-MEM, and ADNI-EF). Longitudinal analyses were performed using mixed model regression with time modeled as days from baseline for each participant. Time was then rescaled so that slopes would represent annual change (days from baseline/

365.25). The time x VEGF interaction term tested whether VEGF levels were associated with change in the given outcome (left hippocampal volume, ADNI-MEM, and ADNI-EF) over the follow-up period. We evaluated the main effects of Aβ-42 and tau in separate models for comparison with VEGF. Correlations among VEGF, CSF Aβ-42, and tau were evaluated using Pearson correlations.

2.5.2 Statistical Analyses: VEGF x CSF Biomarker Interaction on Brain Aging—Next, we evaluated the interaction between VEGF and CSF AD biomarkers (Aβ-42 or total tau) on the three brain aging outcomes to test the neuroprotective effect of VEGF in presence of enhanced AD. Predictors included VEGF level, biomarker level (either Aβ-42 or total tau), and a VEGF x biomarker interaction term. Longitudinal analyses included a time x VEGF x biomarker interaction term to evaluate whether VEGF level interacted with biomarker level in association with change in hippocampal volume, memory, or executive function over the follow-up period. All lower order interactions of this three-way interaction term were included in the model. The CSF AD biomarkers were treated as continuous variables for all analyses, however biomarker groups were also identified for illustration purposes based on previously reported cut-points (Aβ-42 positive ≤ 192, tau positive ≥ 93).[15]

2.5.3 Statistical Analyses: Exploratory Analysis of Diagnosis as an Effect Modifier—Finally, we evaluated all identified significant main effects and interactions of VEGF to evaluate whether the effect of VEGF differed across diagnostic categories. For all models, the NC group was set as the referent.

3. Results

3.1 VEGF Main Effects on Brain Aging

In baseline analyses, increased VEGF was associated with larger hippocampal volume ($t(277)=2.62$, $p=0.009$, Table 2) but was not associated with episodic memory ($t(305)=0.44$, $p=0.66$) or executive function performance ($t(305)=1.38$, $p=0.17$).

In longitudinal analyses, increased VEGF level was associated with less hippocampal atrophy ($t(858)=2.48$, $p=0.01$; Figure 1), less episodic memory decline ($t(1629)=4.09$, $p<0.0001$) and less executive function decline over time ($t(1616)=3.00$, $p=0.003$). In all cases, a high VEGF level was associated with more optimal brain aging.

VEGF was correlated with CSF Aβ-42 ($r=0.22$, $n=279$, $p<0.001$, Figure 1e) and tau ($r = 0.29$, $n=279$, $p<0.001$, Figure 2e).

3.2 VEGF x Aβ-42 Interaction on Brain Aging

At baseline, VEGF did not interact with Aβ-42 in relation to hippocampal volume ($t(274)=0.31$, $p=0.76$), baseline memory performance ($t(302)=-0.06$, $p=0.95$), or baseline executive function performance ($t(302)=0.92$, $p=0.36$).

In longitudinal analyses, there was a VEGF x Aβ-42 interaction in relation to memory performance changes ($t(1618)=-2.53$, $p=0.01$). As seen in Figure 2, a high VEGF level was

associated with better memory performance in the presence of a low Aβ-42 level ("amyloid positive"). There was no VEGF x Aβ-42 interaction in relation to hippocampal atrophy (t(850)=-0.53, p=0.60) or changes in executive function performance over the follow-up interval (t(1605)=-0.58, p=0.56).

3.3 VEGF x Tau Interaction on Brain Aging

At baseline, VEGF did not interact with tau in relation to hippocampal volume (t(271)=-1.11, p=0.27), memory performance (t(299)=1.30, p=0.19), or executive function performance (t(299)=0.62, p=0.54).

In longitudinal analyses, there was a VEGF x tau interaction in relation to hippocampal atrophy (t(845)=4.17, p<0.0001), memory performance changes (t(1610)=2.49, p=0.01), and executive function performance changes (t(1597)=3.71, p=0.0002) across the follow-up interval. As illustrated in Figure 3, in all cases, a high VEGF level was associated with better outcomes in the presence of a higher tau level ("tau positive").

3.4 Diagnosis as an Effect Modifier

At baseline, diagnostic status interacted with VEGF in relation to hippocampal volume (F(2,274)=3.33, p=0.037) whereby the protective effect of VEGF was only apparent in MCI compared to NC. Longitudinally, the observed effects of VEGF did not differ across diagnostic categories. However, given the observed baseline modification of diagnostic status, we present stratified results across all models in the **Supplementary Tables**.

4. Discussion

The present manuscript evaluated whether VEGF relates to reduced neurodegeneration and cognitive decline in older adults. A higher VEGF level was associated with larger baseline hippocampal volume, less hippocampal atrophy over time, and less cognitive decline over time. Interestingly, the neuroprotective effect of VEGF appeared strongest in the presence of enhanced AD biomarkers, consistent with previous reports in Aβ-42 participants,[13] suggesting that angiogenic factors may be particularly important in those individuals showing early hallmarks of the AD cascade. In further support of this theoretical pathway, the baseline effect of VEGF was also strongest in participants with MCI, and when performing stratified analyses, the majority of associations were driven by effects in the MCI group.

The neuroprotective effect of VEGF in CSF is consistent with previous reports that high serum VEGF is associated with a decreased risk for AD,[5] and previous findings in ADNI that VEGF differentiates AD cases from controls.[12] Yet the mechanisms of this effect remain elusive. Our findings add to previous literature associating VEGF with various brain aging outcomes,[13] and suggests that the observed effects may have strong implications for potential interventions. For example, VEGF plays a large role in maintaining neural perfusion homeostasis.[4] In mice, suppressed VEGF levels result in a reduction in perfusion even in the absence of angiogenic deficits.[24] In humans, cerebral hypoperfusion is common in AD,[25] appearing initially in the posterior cingulate and precuneus regions, and later in medial temporal regions including the hippocampus.[26] The protective effects of VEGF, if

mediated through alterations in perfusion, may be particularly beneficial in older adults who are AD biomarker positive before the onset of clinical symptoms. Additional work is needed that teases apart the complex interplay between VEGF and neurodegeneration, particularly targeting mechanisms like neural perfusion, to clarify the pathway of the observed protective effects presented here. Future work leveraging arterial spin labeling MRI data and measures of VEGF would be useful in clarifying the role of cerebral blood flow alterations as a possible mediator of VEGF effects on brain aging.

The observed VEGF-by-biomarker interactions suggest the protective effect of VEGF is strongest in AD biomarker positive individuals, particularly those adults who are tau positive. We observed a robust interaction between VEGF and tau in predicting longitudinal change in hippocampal volume, memory, and executive function. But, more importantly, our results suggest that the effect is not specific to one aspect of AD pathogenesis, as we also observed an interaction between VEGF and Aβ-42 in predicting longitudinal change in memory performance. It is interesting that we did not observe a VEGF x Aβ-42 interaction in relation to hippocampal volume, particularly given the reported protective effect of VEGF in the hippocampus of APP transgenic mice,[6,8,9] and the previously reported effect of VEGF in Aβ-42 positive individuals.[13] Our models treated both VEGF and Aβ-42 as continuous variables rather than binary (positive vs. negative) variables and explicitly tested for an interaction, which may explain the discordant results. We confirmed a strong effect of VEGF in Aβ-42 positive participants in relation to both baseline and longitudinal hippocampal volume (results not shown), so the difference in outcomes between the studies is likely due to the statistical model applied. While interaction effects do explain some of the association between VEGF and neurodegeneration, it appears that there is a strong underlying main effect of VEGF that is present whether biomarker positive or negative.

We also observed an interesting interaction between VEGF and diagnosis whereby the effect of VEGF on baseline hippocampal volume was driven by a strong association in MCI participants. Moreover, when we stratified results across diagnostic categories, the observed effects appeared to be driven primarily by the MCI group, although the study was somewhat underpowered to fully investigate VEGF x Biomarker x Diagnosis interaction models given the sample size and number of model parameters. However, the identified diagnostic interaction and the stratified results certainly suggest that VEGF may have the most relevance in individuals at highest risk for future neurodegeneration.

There are a few potential mechanisms by which VEGF may reduce risk for neurodegeneration. One possibility is that VEGF causes actual reductions in the pathological hallmarks of AD. In support of such an explanation, prior work has demonstrated that treating AD mice with cells secreting VEGF yielded reductions in both tau and amyloid burden at autopsy.[27] Another possibility is that the observed statistical interaction is driven by a physical interaction between VEGF and AD pathology. Prior work has demonstrated that VEGF binds to Aβ-40 and Aβ-42 in *in vitro* experiments, and such binding results in increased neural vulnerability to future insult by reducing the availability of VEGF throughout the brain.[28] That is, in individuals with low levels of VEGF, the binding of VEGF to Aβ-42 could result in a measurable cognitive or neurodegenerative effect due to large net reductions in VEGF activity throughout the brain. Such an effect may not be

observed in individuals with high levels of VEGF at baseline that have sufficient reserves to endure the reduced presence of VEGF throughout the brain as it binds to Aβ-42. It is also possible that non-Aβ-42 sequestration effects, perhaps via VEGF receptors, determine the region-specific expression and activity of VEGF throughout the brain. The current results make it difficult to decipher how VEGF, tau, and Aβ-42 interact at various stages of the AD process, but regardless of the mechanism, VEGF may be particularly relevant to the long-term clinical manifestation of AD in biomarker positive individuals.

This manuscript has several strengths. First, the large sample size makes this study among the largest to evaluate VEGF in humans to date. The availability of CSF data in the ADNI cohort expands prior human work relying on serum VEGF. The longitudinal follow-up period and the combination of cognitive and neuroimaging data allowed us to test for a protective effect of VEGF in relation to several commonly used brain aging phenotypes. Further, the availability of CSF AD biomarker data allowed us to demonstrate that the VEGF protective effect is particularly relevant in AD biomarker positive older adults.

Despite these strengths, a larger sample would have allotted us the opportunity to properly evaluate the VEGF x biomarker interaction across diagnostic groups. Our results suggest the protective effect of VEGF is strongest in the MCI group, but future work is needed to confirm such an observation. Moreover, it would be worthwhile to study VEGF in a cohort with pathological confirmation of AD to evaluate whether the beneficial effect of VEGF is related to a reduction in comorbidities at autopsy, particularly vascular comorbidities that frequently co-occur in clinical AD cases.[29] Such comorbidities are especially relevant to differences in cognitive profiles.[30] We also performed 18 comparisons in this primary analysis, so the possibility of false positives cannot be overlooked. When correcting for multiple comparisons using the Bonferroni procedure, four out of our eight findings remain statistically significant (Table 2). Finally, although CSF tau and Aβ-42 are well established AD biomarkers[15] included in the updated diagnostic criteria used in clinical trials,[31] they are not perfect surrogates for pathological burden at autopsy. Future work should evaluate the interaction between VEGF expression and pathologic burden to clarify the specificity of the interaction effects observed here.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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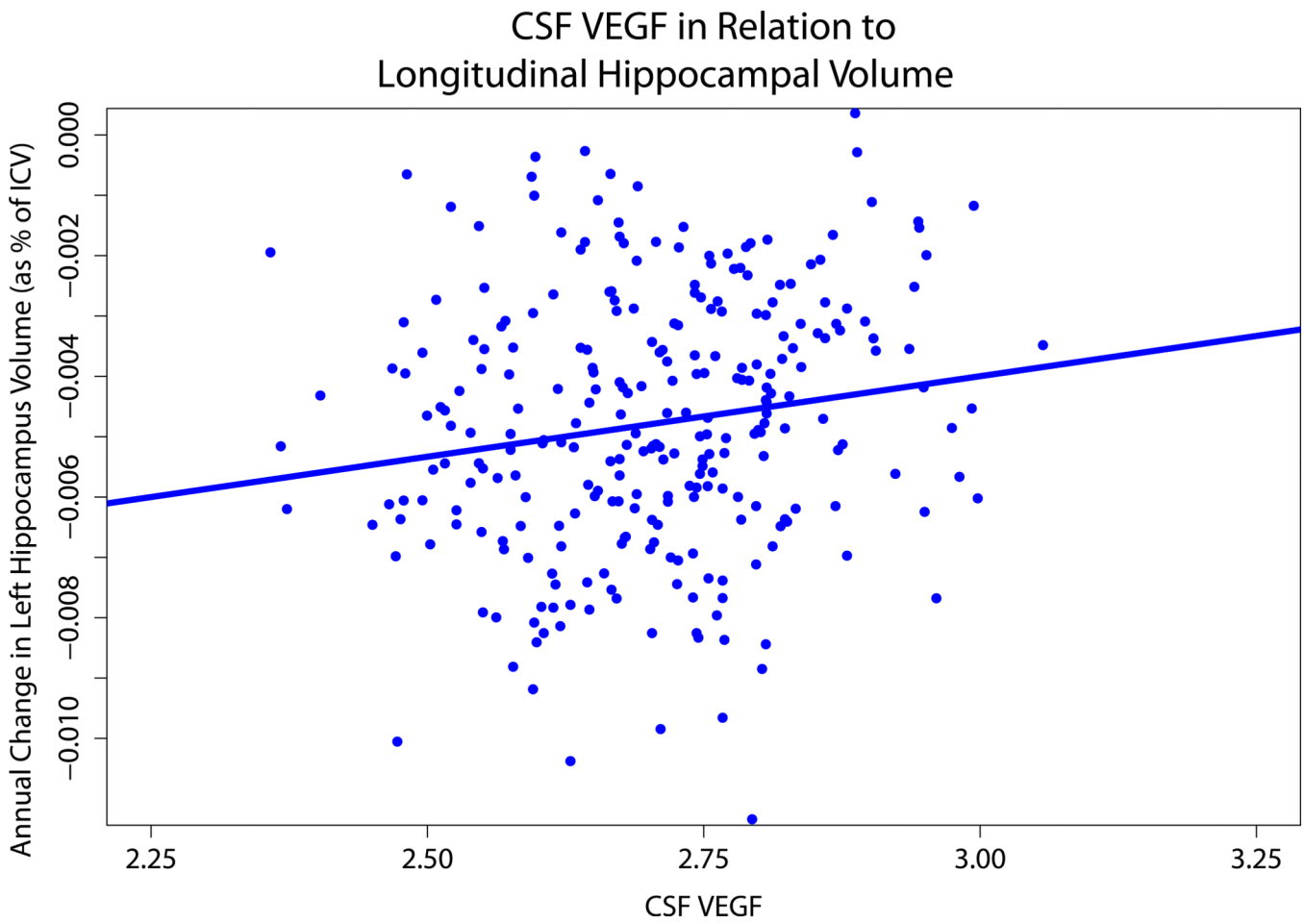


Figure 1. VEGF is associated with hippocampal atrophy

VEGF levels are along the x-axis and left hippocampal volume is along the y axis. Higher levels of VEGF are associated with slower rates of hippocampal atrophy.

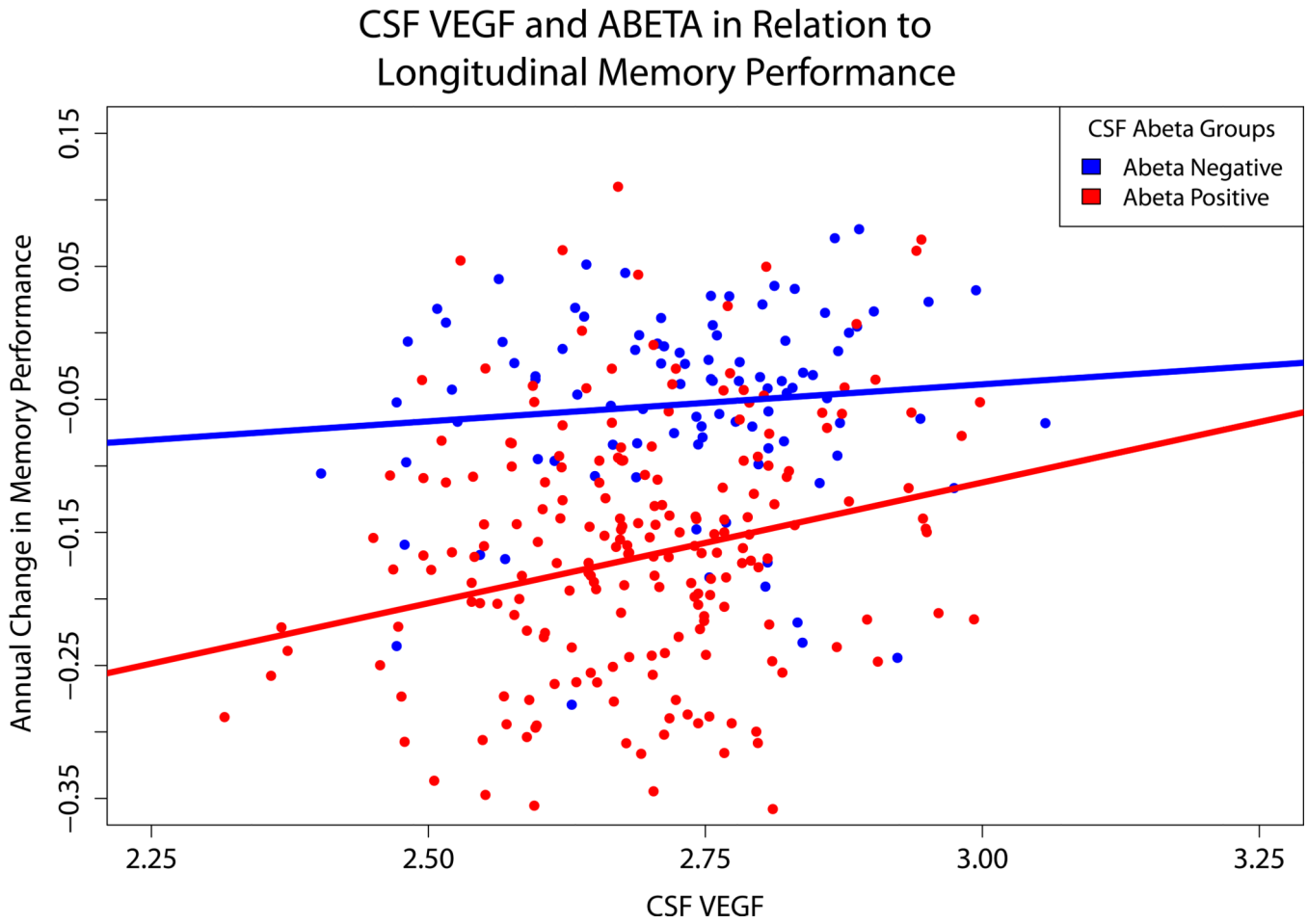
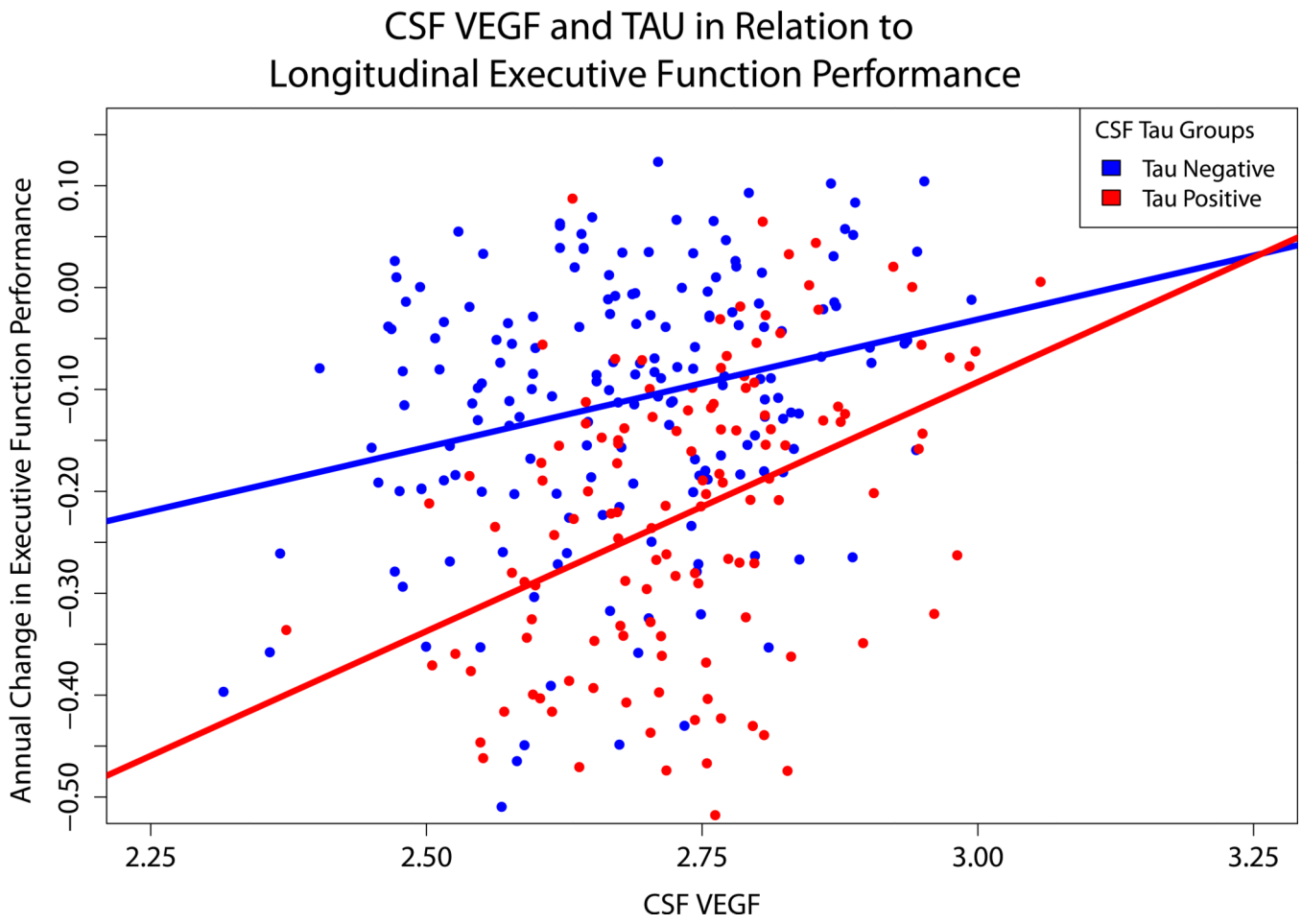


Figure 2. VEGF is associated with longitudinal memory performance in amyloid positive individuals

CSF VEGF levels are along the x-axis and annual change in composite memory performance is along the y axis. Biomarker groups are for illustration only. Amyloid groups are based on a previously identified cut-off value for amyloid positivity (CSF Aβ-42 ≤192).



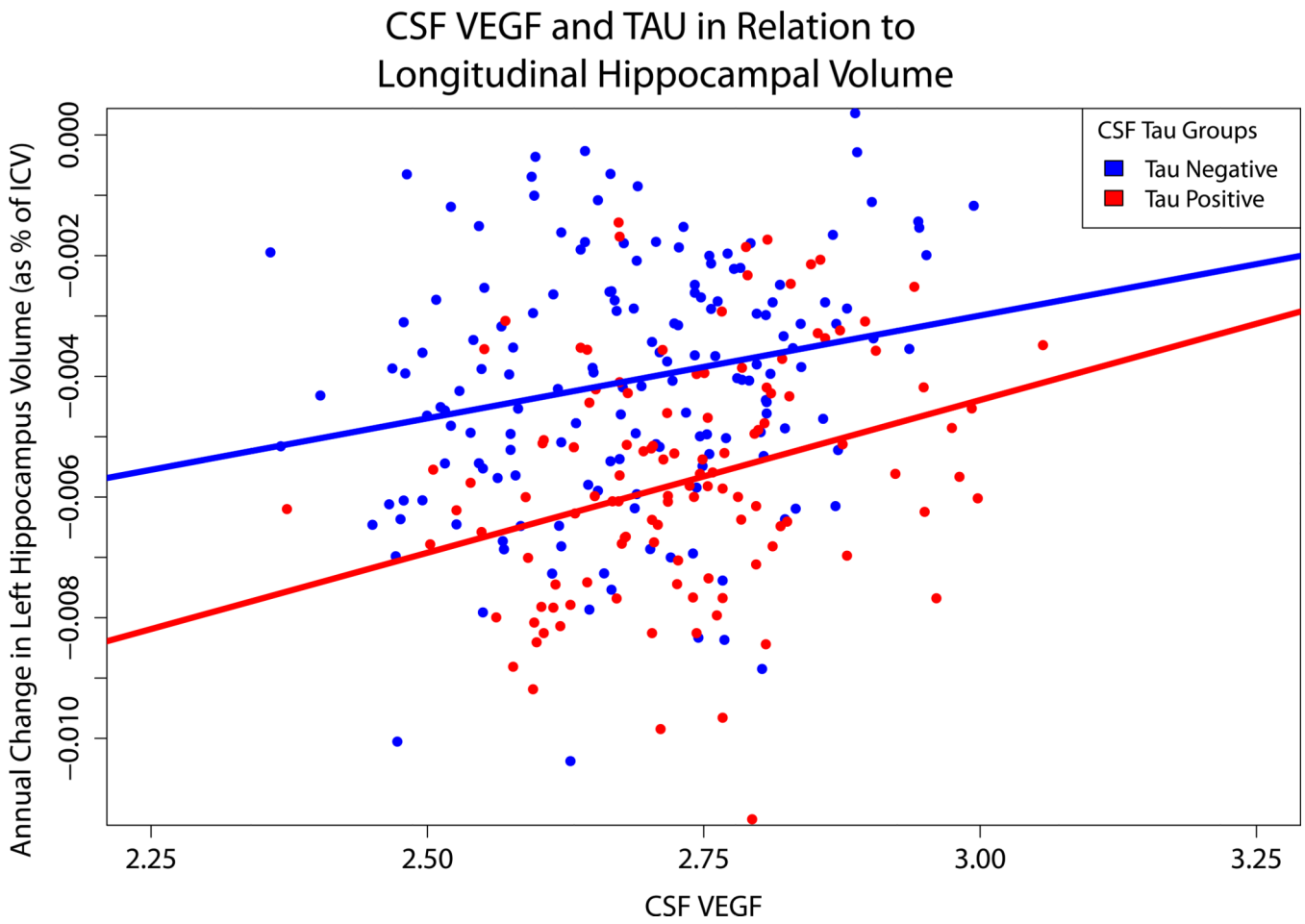


Figure 3. VEGF is associated with longitudinal cognitive performance and longitudinal hippocampal atrophy in tau positive individuals

CSF VEGF levels are along the x-axis. Biomarker groups are for illustration. Tau groups are based on a previously identified cut-off value for tau positivity (Tau Positive >= 93). **Panel A:** Annual change in executive function is along the y axis. **Panel B**: Annual change in hippocampal volume is along the y axis.

Table 1

Sample Characteristics

Brain Volume Dataset	Baseline Clinical Diagnosis[#]		
	Normal Control	Mild Cognitive Impairment	Alzheimer's Disease
Sample Size, n	90	130	59
APOE ε4 Carriers, %	23%	57%	69%
Females, %	50%	32%	46%
Baseline Age, years	76±5	75±7	75±8
Education, years	16±3	16±3	15±3
Visits, total	4.58±2.09	4.48±2.07	2.59±1.10
CSF VEGF, natural log of pg/mL	2.72±0.12	2.71±0.13	2.67±0.13
CSF Total Tau, pg/mL	70±28	105±53	130±61
CSF Aβ-42, pg/mL,	206±56	161±51	143±38
Left Hippocampal Volume (% of Intracranial Volume)[*]	0.24±0.03	0.19±0.03	0.18±0.04
Cognitive Dataset			
Sample Size, n	92	147	67
APOE ε4 Carriers, %	24%	53%	70%
Females, %	50%	32%	43%
Baseline Age, years	76±5	75±7	75±8
Education, years	16±3	16±3	15±3
Visits, total	7.17±1.98	6.71±2.01	3.90±0.35
CSF VEGF, natural log of pg/mL	2.72±0.12	2.71±0.13	2.66±0.13
CSF Total Tau, pg/mL	69±27	104±52	124±60
CSF Aβ-42, pg/mL	206±56	161±52	142±37
Memory, z-score	0.96±0.52	−0.16±0.56	−0.88±0.55
Executive Function, z-score	0.67±0.61	−0.09±0.71	−1.01±0.81

[#]Diagnostic groups were defined according to the ADNI protocol. Normal Control subjects had a Mini-Mental Status Examination (MMSE) score between 24 and 30, a Clinical Dementia Rating (CDR) score of 0, and were not depressed (Geriatric Depression Scale score <6). Mild Cognitive Impairment subjects had a MMSE score between 24 and 30, objective memory impairment, subjective memory impairment, and a CDR score of 0.5. Alzheimer's Disease subjects met clinical criteria for dementia, had an MMSE of between 20 and 26, and had CDR score of .5 or 1.

[*]ICV corrected values are for illustration purposes only, however ICV was entered into all statistical models as a covariate.

Table 2

Associations Between VEGF and Brain Aging Variables

	VEGF		**TAU**		**Aβ-42**		**VEGF x Aβ-42**		**VEGF x Tau**	
Cross-Sectional Outcomes	β	p-value	β	p-value	β	p-value	β	p-value	β	p-value
Hippocampal Volume	567	0.009#	−0.71	0.213	1.13	0.032	1.17	0.757	−4.48	0.268
Episodic Memory Composite	0.11	0.661	−0.001	0.078	0.002	0.003	−0.0002	0.951	0.006	0.195
Executive Function Composite	0.44	0.169	−0.002	0.015	0.001	0.066	0.005	0.359	0.004	0.539
Longitudinal Outcomes	β	p-value	β	p-value	β	p-value	β	p-value	β	p-value
Hippocampal Volume	78.76	0.013	−0.41	4.2×10^{-7}	0.46	2.1×10^{-12}	−0.27	0.597	2.98	3.3×10^{-5}*
Episodic Memory Composite	0.26	4.6×10^{-5}*	−0.001	4.5×10^{-11}	0.001	4.1×10^{-13}	−0.002	0.011	0.003	0.013
Executive Function Composite	0.27	0.00277*	−0.001	3.5×10^{-11}	0.001	6.0×10^{-16}	−0.0008	0.565	0.007	0.0002*

Boldface signifies effects that are significant at $p < 0.05$.

*Signifies effect is significant when correcting for multiple comparisons (Bonferroni).

#Signifies a diagnosis interaction ($p < .05$)