

## Original Investigation

# The Role of Vascular Endothelial Growth Factor in Neurodegeneration and Cognitive Decline

## Exploring Interactions With Biomarkers of Alzheimer Disease

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**IMPORTANCE** A subset of older adults present post mortem with Alzheimer 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** To evaluate whether VEGF levels are associated with brain aging outcomes (hippocampal volume and cognition) and to further evaluate whether VEGF modifies relations between AD biomarkers and brain aging outcomes.

**DESIGN, SETTING, AND PARTICIPANTS** Biomarker analysis using neuroimaging and neuropsychological outcomes from the Alzheimer's Disease Neuroimaging Initiative. This prospective longitudinal study across North America included individuals with normal cognition (n = 90), mild cognitive impairment (n = 130), and AD (n = 59) and began in October 2004, with follow-up ongoing.

**MAIN OUTCOMES AND MEASURES** Cerebrospinal fluid VEGF was cross-sectionally related to brain aging outcomes (hippocampal volume, episodic memory, and executive function) using a general linear model and longitudinally using mixed-effects regression. Alzheimer disease biomarker (cerebrospinal fluid  $\beta$ -amyloid 42 and total tau)-by-VEGF interactions evaluated the effect of VEGF on brain aging outcomes in the presence of enhanced AD biomarkers.

**RESULTS** Vascular endothelial growth factor was associated with baseline hippocampal volume ( $t_{277} = 2.62$ ;  $P = .009$ ), longitudinal hippocampal atrophy ( $t_{858} = 2.48$ ;  $P = .01$ ), and longitudinal decline in memory ( $t_{1629} = 4.09$ ;  $P < .001$ ) and executive function ( $t_{1616} = 3.00$ ;  $P = .003$ ). Vascular endothelial growth factor interacted with tau in predicting longitudinal hippocampal atrophy ( $t_{845} = 4.17$ ;  $P < .001$ ), memory decline ( $t_{1610} = 2.49$ ;  $P = .01$ ), and executive function decline ( $t_{1597} = 3.71$ ;  $P < .001$ ). Vascular endothelial growth factor interacted with  $\beta$ -amyloid 42 in predicting longitudinal memory decline ( $t_{1618} = -2.53$ ;  $P = .01$ ).

**CONCLUSIONS AND RELEVANCE** Elevated cerebrospinal fluid 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.

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

Vascular endothelial growth factor has also been evaluated as a potential biomarker for AD, although 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 control individuals (ie, no neurological disease or deficit).<sup>10</sup> A second study found that CSF VEGF levels did not differ between AD and cognitively normal control individuals, further confounding the issue.<sup>11</sup> Data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) appears to be more consistent with the serum results previously reported<sup>5</sup> and found that lower levels of VEGF in CSF distinguish AD from healthy control individuals with 76% sensitivity and 84% specificity.<sup>12</sup>

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 older individuals at risk for AD. One study evaluated more than 80 CSF analytes in relation to brain aging outcomes and found that lower levels of CSF VEGF were related to smaller hippocampi, larger ventricles, and faster decline on the Mini-Mental State Examination over 12 months.<sup>13</sup> 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, and 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 study conducted 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 2 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 out-

comes differed between cognitive diagnostic categories. Finally, we tested the interaction between VEGF and continuous measures of CSF AD biomarkers ( $A\beta$ -42 and 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 and cognitive decline) would not be specific to one pathologic process but would be strongest in the presence of either AD biomarker ( $A\beta$ -42 or total tau).

## Methods

Participants were drawn from the ADNI launched in October 2004 (<http://adni.loni.ucla.edu/>). The original ADNI study enrolled approximately 800 participants aged 55 to 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 the Vanderbilt University Medical Center institutional review board prior to data analysis.

### Participants

We accessed publicly available data from the ADNI on June 1, 2014. Participants were enrolled in the ADNI based on criteria outlined in the ADNI protocol (<http://www.adni-info.org/Scientists/ADNIOverview.aspx>). For the present analyses, we included all participants with CSF multiplex data that passed ADNI's quality-control procedures (defined further on), CSF measurement of  $A\beta$ -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 1.5-T magnetic resonance imaging 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**.

### CSF Analyte and Biomarker Processing

The ADNI CSF protocol, including the quantification of  $A\beta$ -42 and total tau, has been detailed elsewhere.<sup>14,15</sup> For the present analyses, we compiled a data set across the UPENN1-UPENN5 data sources available for download and used the first measure of total tau and  $A\beta$ -42 available for each participant.

Vascular endothelial growth factor was calculated as part of a CSF multiplex proteomic processing stream using an xMAP multiplex panel (MyriadRBM),<sup>16</sup> 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 quality control if the test-retest sample was less than 7, if the mean percentage difference was greater than 35%, if the mean absolute percentage difference was greater than 60%, or if the Bland-Altman slope and inter-

Table 1. Sample Characteristics

Characteristic	Baseline Clinical Diagnosis, Mean (SD) <sup>a</sup>		
	Normal Control	Mild Cognitive Impairment	Alzheimer Disease
<b>Brain volume data set</b>			
Sample size, No.	90	130	59
APOE ε4 carriers, %	23	57	69
Female, %	50	32	46
Baseline age, y	76 (5)	75 (7)	75 (8)
Education, y	16 (3)	16 (3)	15 (3)
Total visits	4.58 (2.09)	4.48 (2.07)	2.59 (1.10)
<b>CSF, pg/mL</b>			
VEGF, natural log	2.72 (0.12)	2.71 (0.13)	2.67 (0.13)
Total tau	70 (28)	105 (53)	130 (61)
Aβ-42	206 (56)	161 (51)	143 (38)
Left hippocampal volume (% of intracranial volume) <sup>b</sup>	0.24 (0.03)	0.19 (0.03)	0.18 (0.04)
<b>Cognitive data set</b>			
Sample size, No.	92	147	67
APOE ε4 carriers, %	24	53	70
Female, %	50	32	43
Baseline age, y	76 (5)	75 (7)	75 (8)
Education, y	16 (3)	16 (3)	15 (3)
Total visits	7.17 (1.98)	6.71 (2.01)	3.90 (0.35)
<b>CSF, pg/mL</b>			
VEGF, natural log	2.72 (0.12)	2.71 (0.13)	2.66 (0.13)
Total tau	69 (27)	104 (52)	124 (60)
Aβ-42	206 (56)	161 (52)	142 (37)
<b>z Score</b>			
Memory	0.96 (0.52)	-0.16 (0.56)	-0.88 (0.55)
Executive function	0.67 (0.61)	-0.09 (0.71)	-1.01 (0.81)

Abbreviations: Aβ-42; β-amyloid 42; APOE, apolipoprotein E; CSF, cerebrospinal fluid; VEGF, vascular endothelial growth factor.

<sup>a</sup> Diagnostic groups were defined according to the Alzheimer's Disease Neuroimaging Initiative protocol. Normal control individuals had a Mini-Mental State Examination (MMSE) score between 24 and 30, a Clinical Dementia Rating (CDR) score of 0, and were not depressed (Geriatric Depression scale score <6). Patients with mild cognitive impairment had an MMSE score between 24 and 30, objective memory impairment, subjective memory impairment, and a CDR score of 0.5. Patients with Alzheimer disease met clinical criteria for dementia, had an MMSE score of between 20 and 26, and had a CDR score of 0.5 or 1.

<sup>b</sup> Intracranial volume-corrected values are for illustration purposes only; however, intracranial volume was entered into all statistical models as a covariate.

cept 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 quality-control procedures.

### Neuropsychological Composites

The ADNI neuropsychological protocol, including calculation of episodic memory and executive function composite measures, has been reported previously.<sup>17,18</sup> We leveraged a memory (ADNI-MEM) and executive function (ADNI-EF) composite score in the present analyses. The 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. The 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.

### Quantification of Hippocampal Volume and Hippocampal Atrophy

The ADNI neuroimaging protocol has been reported in detail elsewhere.<sup>19</sup> Images for the current study included original uncorrected 1.5-T T1-weighted high-resolution 3-dimensional structural data. Cortical reconstruction and volumetric seg-

mentation were performed with the FreeSurfer image analysis suite version 4.3 (<http://surfer.nmr.mgh.harvard.edu/>).<sup>20-22</sup> FreeSurfer processing in the ADNI has been described previously.<sup>23</sup> An early version of the longitudinal image-processing framework was used to process the sequential scans.<sup>24</sup> Left hippocampal volume was the primary outcome measurement and intracranial volume was included as a covariate in all volumetric analyses, both of which were defined by FreeSurfer.<sup>25</sup>

### 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 intracranial volume (for neuroimaging analyses). Significance was set a priori as  $\alpha = .05$ .

### VEGF Main Effects on Brain Aging

Baseline effects were estimated using a general linear model for each of the 3 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-by-VEGF interaction term tested whether VEGF levels were associated with change in the given out-

Table 2. Associations Between VEGF and Brain Aging Variables<sup>a</sup>

Variable	VEGF		Tau		A $\beta$ -42		VEGF $\times$ A $\beta$ -42		VEGF $\times$ Tau	
	$\beta$	P Value	$\beta$	P Value	$\beta$	P Value	$\beta$	P Value	$\beta$	P Value
Cross-sectional outcomes										
Hippocampal volume	567	.009 <sup>b</sup>	-0.71	.21	1.13	.03	1.17	.76	-4.48	.27
Episodic memory composite	0.11	.66	-0.001	.08	0.002	.003	-0.0002	.95	0.006	.20
Executive function composite	0.44	.17	-0.002	.02	0.001	.07	0.005	.36	0.004	.54
Longitudinal outcomes										
Hippocampal volume	78.76	.01	-0.41	$4.2 \times 10^{-7}$	0.46	$2.1 \times 10^{-12}$	-0.27	.60	2.98	$3.3 \times 10^{-5c}$
Episodic memory composite	0.26	$4.6 \times 10^{-5c}$	-0.001	$4.5 \times 10^{-11}$	0.001	$4.1 \times 10^{-13}$	-0.002	.01	0.003	.01
Executive function composite	0.27	.003 <sup>c</sup>	-0.001	$3.5 \times 10^{-11}$	0.001	$6.0 \times 10^{-16}$	-0.0008	.57	0.007	<.001 <sup>c</sup>

Abbreviations: A $\beta$ -42;  $\beta$ -amyloid 42; VEGF, vascular endothelial growth factor.

<sup>a</sup> Boldface signifies effects that are significant at  $P < .05$ .

<sup>b</sup> Signifies a diagnosis interaction ( $P < .05$ ).

<sup>c</sup> Signifies effect is significant when correcting for multiple comparisons (Bonferroni).

come (left hippocampal volume, ADNI-MEM, and ADNI-EF) over the follow-up period. We evaluated the main effects of A $\beta$ -42 and tau in separate models for comparison with VEGF. Correlations among VEGF, CSF A $\beta$ -42, and tau were evaluated using Pearson correlations.

#### VEGF-by-CSF Biomarker Interaction on Brain Aging

Next, we evaluated the interaction between VEGF and CSF AD biomarkers (A $\beta$ -42 or total tau) on the 3 brain aging outcomes to test the neuroprotective effect of VEGF in the presence of enhanced AD. Predictors included VEGF level, biomarker level (either A $\beta$ -42 or total tau), and a VEGF-by-biomarker interaction term. Longitudinal analyses included a time-by-VEGF-by-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 3-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 (A $\beta$ -42 positive  $\leq 192$  and tau positive  $\geq 93$  pg/mL).<sup>15</sup>

#### Exploratory Analysis of Diagnosis as an Effect Modifier

Finally, we evaluated all identified significant main effects and interactions of VEGF to determine whether the effect of VEGF differed across diagnostic categories. For all models, the normal cognition group was set as the referent.

## Results

### VEGF Main Effects on Brain Aging

In baseline analyses, increased VEGF was associated with larger hippocampal volume ( $t_{277} = 2.62$ ;  $P = .009$ ; Table 2) but was not associated with episodic memory ( $t_{305} = 0.44$ ;  $P = .66$ ) or executive function performance ( $t_{305} = 1.38$ ;  $P = .17$ ).

In longitudinal analyses, increased VEGF level was associated with less hippocampal atrophy ( $t_{858} = 2.48$ ;  $P = .01$ ; Figure 1), less episodic memory decline ( $t_{1629} = 4.09$ ;  $P < .001$ ), and less executive function decline over time ( $t_{1616} = 3.00$ ;  $P = .003$ ). In all cases, a high VEGF level was associated with more optimal brain aging.

Vascular endothelial growth factor was correlated with CSF A $\beta$ -42 ( $r = 0.22$ ;  $n = 279$ ;  $P < .001$ ; eFigure 1 in the Supplement) and tau ( $r = 0.29$ ;  $n = 279$ ;  $P < .001$ ; eFigure 2 in the Supplement).

### VEGF-by-A $\beta$ -42 Interaction on Brain Aging

At baseline, VEGF did not interact with A $\beta$ -42 in relation to hippocampal volume ( $t_{274} = 0.31$ ;  $P = .76$ ), baseline memory performance ( $t_{302} = -0.06$ ;  $P = .95$ ), or baseline executive function performance ( $t_{302} = 0.92$ ;  $P = .36$ ).

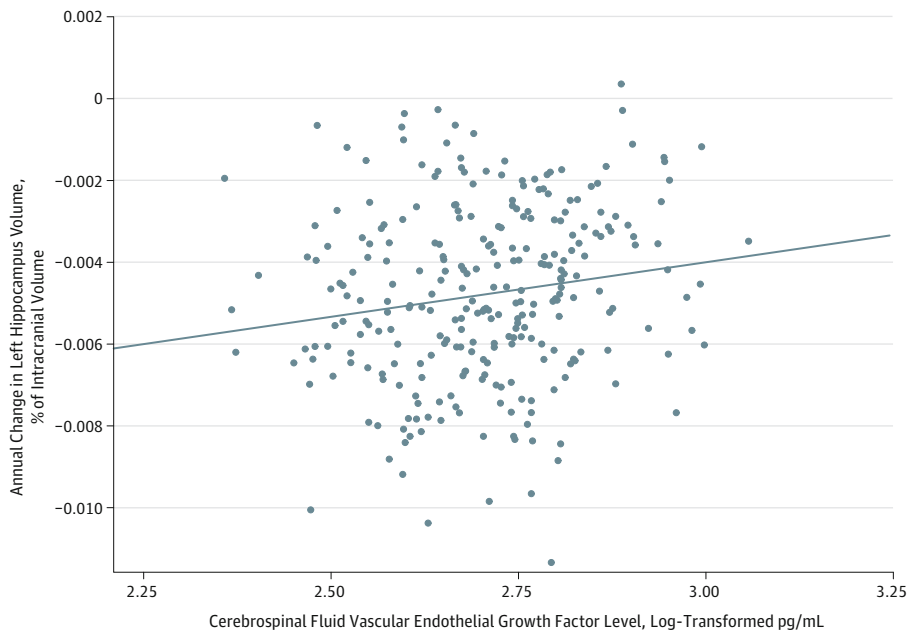
In longitudinal analyses, there was a VEGF-by-A $\beta$ -42 interaction in relation to memory performance changes ( $t_{1618} = -2.53$ ;  $P = .01$ ). As seen in Figure 2, a high VEGF level was associated with better memory performance in the presence of a low A $\beta$ -42 level (amyloid positive). There was no VEGF-by-A $\beta$ -42 interaction in relation to hippocampal atrophy ( $t_{850} = -0.53$ ;  $P = .60$ ) or changes in executive function performance over the follow-up interval ( $t_{1605} = -0.58$ ;  $P = .56$ ).

### VEGF-by-Tau Interaction on Brain Aging

At baseline, VEGF did not interact with tau in relation to hippocampal volume ( $t_{271} = -1.11$ ;  $P = .27$ ), memory performance ( $t_{299} = 1.30$ ;  $P = .19$ ), or executive function performance ( $t_{299} = 0.62$ ;  $P = .54$ ).

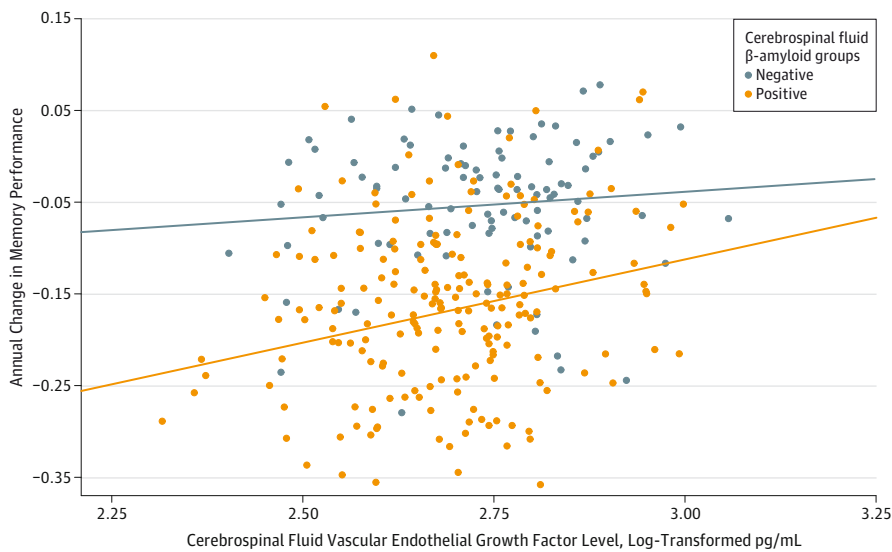
In longitudinal analyses, there was a VEGF-by-tau interaction in relation to hippocampal atrophy ( $t_{845} = 4.17$ ;  $P < .001$ ), memory performance changes ( $t_{1610} = 2.49$ ;  $P = .01$ ), and executive function performance changes ( $t_{1597} = 3.71$ ;  $P < .001$ ) across the follow-up interval. 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).

Figure 1. Association Between Vascular Endothelial Growth Factor and Hippocampal Atrophy



Higher levels of vascular endothelial growth factor are associated with slower rates of hippocampal atrophy.

Figure 2. Association Between Vascular Endothelial Growth Factor and Longitudinal Memory Performance in Amyloid-Positive Individuals



Biomarker groups are for illustration only. Amyloid groups are based on a previously identified cutoff value for amyloid positivity (cerebrospinal fluid  $\beta$ -amyloid  $42 \leq 192$  pg/mL).

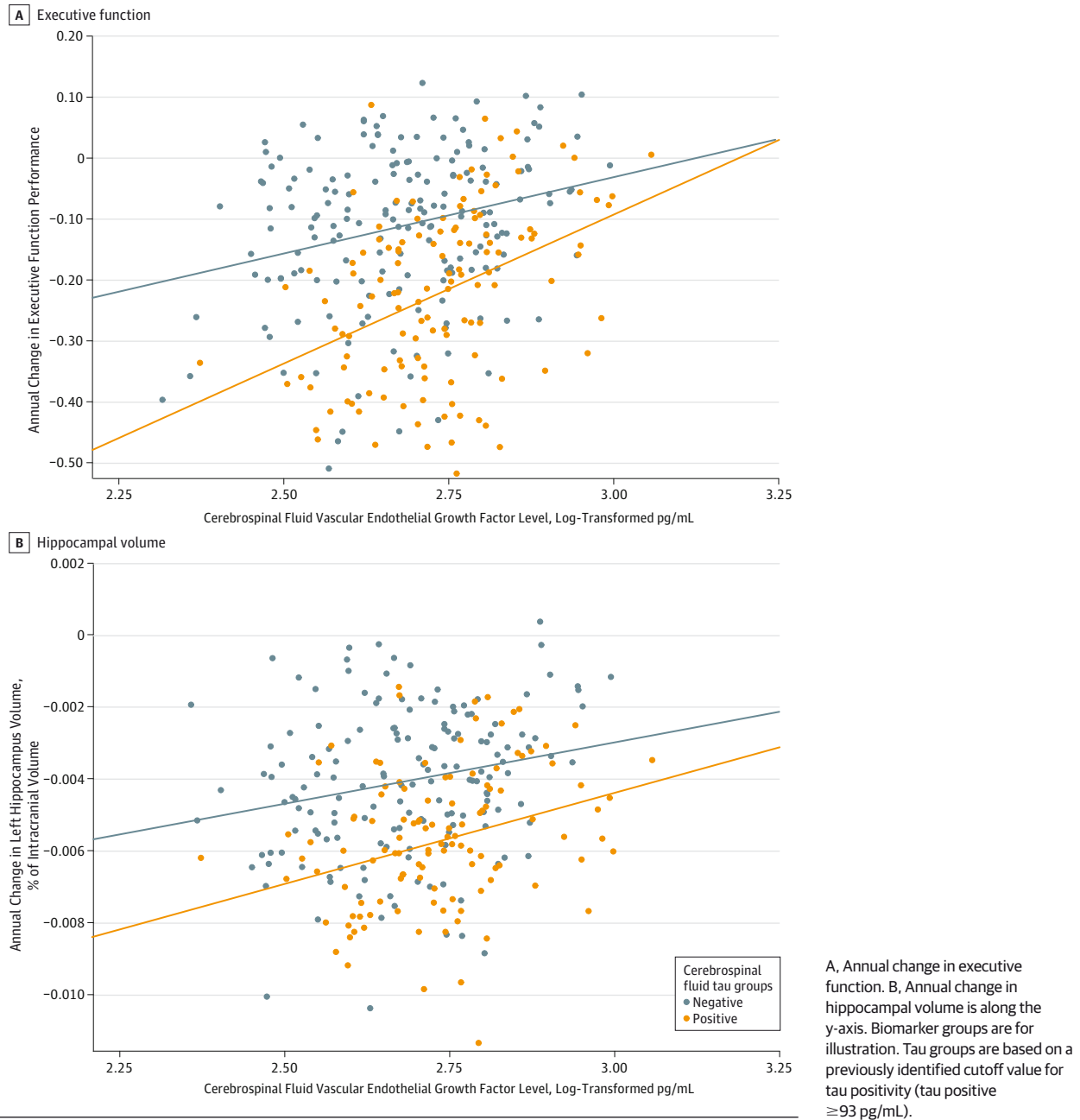
**Diagnosis as an Effect Modifier**

At baseline, diagnostic status interacted with VEGF in relation to hippocampal volume ( $F_{2,274} = 3.33$ ;  $P = .04$ ), whereby the protective effect of VEGF was only apparent in those individuals with mild cognitive impairment (MCI) compared with normal cognition individuals. 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 eTables 1 through 3 in the Supplement.

**Discussion**

The present study 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

**Figure 3. Association Between Vascular Endothelial Growth Factor and Longitudinal Cognitive Performance and Longitudinal Hippocampal Atrophy in Tau-Positive Individuals**



$A\beta$ -42 participants,<sup>13</sup> 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, most 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<sup>5</sup> and previous findings in the ADNI that VEGF differentiates AD cases from control individuals.<sup>12</sup> Yet the mechanisms of this effect remain elusive. Our findings add to

previous literature associating VEGF with various brain aging outcomes<sup>13</sup> and suggest that the observed effects may have strong implications for potential interventions. For example, VEGF plays a large role in maintaining neural perfusion homeostasis.<sup>4</sup> In mice, suppressed VEGF levels result in a reduction in perfusion even in the absence of angiogenic deficits.<sup>26</sup> In humans, cerebral hypoperfusion is common in AD,<sup>27</sup> appearing initially in the posterior cingulate and precuneus regions and later in medial temporal regions including the hippocampus.<sup>28</sup> 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, such as neural perfusion, to clarify the pathway of the observed protective effects presented here. Future work leveraging arterial spin labeling-magnetic resonance imaging 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. However, more importantly, our results suggested that the effect is not specific to one aspect of AD pathogenesis, as we also observed an interaction between VEGF and A $\beta$ -42 in predicting longitudinal change in memory performance. It is interesting that we did not observe a VEGF-by-A $\beta$ -42 interaction in relation to hippocampal volume, particularly given the reported protective effect of VEGF in the hippocampus of *APP* transgenic mice,<sup>6,8,9</sup> and the previously reported effect of VEGF in A $\beta$ -42-positive individuals.<sup>13</sup> Our models treated both VEGF and A $\beta$ -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 A $\beta$ -42-positive participants in relation to both baseline and longitudinal hippocampal volume (results not shown); therefore, the difference in outcomes between the studies is likely owing 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 participants with MCI. 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 the VEGF-by-biomarker-by-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 the 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.<sup>29</sup> Another possibility is that the observed statistical interaction is driven by a physical interaction between VEGF and AD pa-

thology. Prior work has demonstrated that VEGF binds to A $\beta$ -40 and A $\beta$ -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.<sup>30</sup> That is, in individuals with low levels of VEGF, the binding of VEGF to A $\beta$ -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 who have sufficient reserves to endure the reduced presence of VEGF throughout the brain as it binds to A $\beta$ -42. It is also possible that non-A $\beta$ -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 A $\beta$ -42 interact at various stages of the AD process; however, regardless of the mechanism, VEGF may be particularly relevant to the long-term clinical manifestation of AD in biomarker-positive individuals.

## Conclusions

This study had several strengths. First, the large sample size made 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 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-by-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.<sup>31</sup> Such comorbidities are especially relevant to differences in cognitive profiles.<sup>32</sup> 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, 4 of our 8 findings remained statistically significant (Table 2). Finally, although CSF tau and A $\beta$ -42 are well-established AD biomarkers<sup>15</sup> included in the updated diagnostic criteria used in clinical trials,<sup>33</sup> 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.

### ARTICLE INFORMATION

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responsibility for the integrity of the data and the accuracy of the data analysis.

*Study concept and design:* All authors.

*Acquisition, analysis, or interpretation of data:* All authors.

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*Statistical analysis:* Hohman.

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