

CSF VEGF Was Positively Associated with Neurogranin Independent of β -Amyloid Pathology

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Yangping Huang
Jun Wang
Bihong Zhu
Pan Fu

Department of Neurology, Taizhou First
People's Hospital, Taizhou, Zhejiang,
People's Republic of China

Purpose: Increasing evidence suggests that both vascular endothelial growth factor (VEGF) and synaptic failure have been involved in the pathogenesis of Alzheimer's disease (AD). However, it is not clear whether CSF VEGF levels are associated with synaptic function in living human.

Patients and Methods: In the present study, we included a total of 291 older individuals, including 83 individuals with normal cognition (NC), 143 individuals with mild cognitive impairment (MCI) and 65 patients with AD. Several linear regression models were conducted to examine the associations of CSF VEGF with CSF neurogranin levels (NG, reflecting synaptic degeneration) when controlling for other potential confounding factors, including age, gender, years of education, clinical diagnosis, APOE4 genotype and CSF β -amyloid 42 ($A\beta$ 42) levels.

Results: There was no significant difference in VEGF levels between the three diagnostic groups. In the pooled sample, females had significantly lower levels of VEGF than males. $A\beta$ -positive (CSF $A\beta$ 42 < 192 pg/mL) individuals had lower levels of VEGF than $A\beta$ -negative individuals. However, the relationships between VEGF and NG levels were not modified by disease stage. Finally, we found that CSF VEGF levels were associated with NG levels with adjustment of age, gender, years of education, clinical diagnosis, APOE4 genotype and CSF $A\beta$ 42 levels.

Conclusion: CSF VEGF levels were associated with NG independent of AD pathology and disease stage.

Keywords: vascular endothelial growth factor, neurogranin, synaptic dysfunction, Alzheimer's disease

Introduction

Synaptic failure has been implicated as an important feature of Alzheimer's disease (AD) pathophysiology.¹ Neurogranin (NG), a postsynaptic protein, was reported to be increased in the CSF of patients with mild cognitive impairment (MCI) and AD compared to cognitively normal older people.²⁻⁴ In addition, other studies indicated that elevated CSF NG levels may be specific for AD.⁵⁻⁷ Recent studies found that higher levels of NG in CSF can predict faster rates of cognitive decline in MCI and progression from MCI to AD.⁸ Thus, NG appears to be a promising biomarker reflecting AD-related synaptic degeneration.

Vascular endothelial growth factor (VEGF) plays crucial roles in modulation of vascular remodeling, permeability, angiogenesis, repair and inflammation.⁹ Recent studies revealed that VEGF is also involved in the pathogenesis of AD. For example, preclinical studies revealed that the transplantation of VEGF-overexpressing stem cells or VEGF-releasing nanospheres to AD mice improves cognitive deficits and reduces amyloid deposition.^{10,11} Additionally, several longitudinal prospective studies

Correspondence: Pan Fu
Email fp7154515@163.com

suggested that increased VEGF levels in CSF are associated with reduced hippocampal atrophy and less cognitive decline,^{12,13} suggesting that VEGF may be neuroprotective. However, it is not clear whether levels of VEGF in CSF are associated with NG (reflecting synaptic degeneration) in the living human brain.

In the present study, we aimed to examine the associations of CSF VEGF levels with NG in individuals with normal cognition (NC), individuals with MCI and AD from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. These findings might provide critical insight into the neuropathological mechanisms by which VEGF affects AD.

Patients and Methods

Alzheimer's Disease Neuroimaging Initiative Study

Data used for this analysis were extracted from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (<http://adni.loni.usc.edu>). The ADNI study was launched in 2003 with the primary goal of investigating whether neuropsychological assessments, serial MRI, PET and other biomarkers can be integrated to predict the decline of cognition. The ADNI centers obtained local institutional review board approval, and all subjects provided written informed consent. Further information can be found at the ADNI website (<http://adni.loni.usc.edu>).

Participants

We included individuals who had baseline CSF VEGF and NG samples. In this study, there were 83 individuals with NC, 143 individuals with MCI and 65 patients with mild AD dementia. Individuals with NC had a minimal state examination (MMSE) score of 24 or higher and a clinical dementia rating (CDR) score of 0. Individuals with MCI had a subjective memory complaint, a CDR score of 0, a MMSE score ranging between 24 and 30, an objective verbal memory impairment as examined by Wechsler Memory Scale Logical memory II, largely preserved activities of daily living, and absence of dementia. Patients with mild AD met the NINCDS/ADRDA criteria for probable AD, and had a CDR score of 1 or lower and a MMSE score ranging from 20 to 26.

Measurement of VEGF in CSF

VEGF levels were measured as part of a CSF multiplex proteomic processing stream using an xMAP multiplex panel (MyriadRBM), details of which can be found at the

ADNI website (<http://adni.loni.usc.edu/wp-content/uploads/2012/01/2011Dec28-Biomarkers-Consortium-DataPrimer-FINAL1.pdf>). In brief, analytes were removed if they can not pass the ANDI quality-control procedures: (1) the test-retest sample was ≥ 7 ; (2) the mean percentage difference was $\leq 35\%$; (3) the mean absolute percentage difference was $\leq 60\%$. The VEGF analyte successfully passed all these procedures. Values are given in pg/mL. The data were log transformed before statistical analysis.

Measurement of NG in CSF

Levels of NG in CSF were measured using electrochemiluminescence technology (Meso Scale Discovery, Gaithersburg, Maryland, USA) using Ng 7 (a monoclonal antibody specific for Ng) as a coating antibody and polyclonal NG anti-rabbit (ab 23,570, Upstate) as a detector antibody.¹⁴ The information about the measurement of NG in CSF has been detailed elsewhere.¹⁵

Measurement of CSF A β 42 Levels

Levels of A β 42 in CSF were measured using xMAP Luminex platform and Innogenetics/Fujirebio AlzBio3 immunoassay, which has been detailed elsewhere.¹⁶ Values are given in pg/mL. A β status was defined based on a previously established cut-off point (A β +: CSF A β 42 ≤ 192 pg/mL; A β -: CSF A β 42 > 192 pg/mL).¹⁷

Statistical Analysis

The one-way ANOVA was conducted to investigate the differences in continuous variables, and the Pearson χ^2 was applied to compare the distributions of categorical parameters among the three groups. The Pearson correlation test was performed to examine the relationship between CSF VEGF and NG levels in the pooled sample and within each diagnostic group. Finally, several linear regression models were used to investigate the associations between CSF VEGF and NG levels in the pooled sample: model 1 was unadjusted; model 2 was adjusted for age, gender, educational attainment, APOE4 genotype and clinical diagnosis; model 3 was additionally adjusted for A β status (A β -negative vs A β -positive). All data analyses were conducted using R software. The level of statistical significance was set at $p < 0.05$.

Results

Demographic and Clinical Characteristics

In this analysis, a total of 291 participants were included: 83 individuals with NC, 143 individuals with MCI and

65 patients with mild AD dementia (Table 1). There were no significant differences in age and educational attainment among the three groups. Expectedly, a significant difference in MMSE score across the three groups was observed (NC > MCI > AD, $p < 0.001$). Consistent with previous studies, there were significant differences in CSF A β 42 levels across the three groups (A β 42: NC > MCI > AD, $p < 0.001$).

CSF VEGF Levels in the Three Groups

As shown in Table 1 and Figure 1, no significant differences in CSF VEGF levels were found across the three groups ($p = 0.06$).

Relationships Between CSF VEGF and Other Variables in the Pooled Sample

As shown in Figure 2, CSF VEGF was associated with age ($r = 0.24$, $p < 0.001$) and MMSE scores ($r = 0.16$, $p = 0.006$) in the pooled sample. However, no relationship between VEGF and years of education was observed ($r = 0.012$, $p = 0.84$). Compared with males, females had significant lower levels of VEGF (Females: 2.68 ± 0.11 ; Males: 2.73 ± 0.13 ; $t = 3.4$, $p < 0.001$). A β ⁺ individuals had lower levels of VEGF than A β ⁻ individuals (A β ⁺: 2.7 ± 0.12 ; A β ⁻: 2.75 ± 0.12 ; $t = 3.4$, $p < 0.001$). However, no significant difference in VEGF between APOE4 carriers and non-carriers was observed ($p > 0.05$).

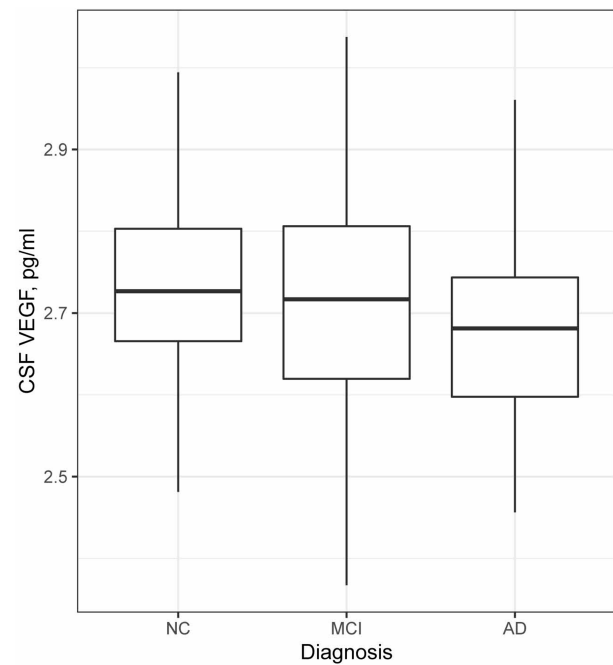


Figure 1 CSF VEGF levels in the three groups. No significant differences in CSF VEGF levels were found across the three groups ($p = 0.06$).

Abbreviations: NC, normal controls; MCI, mild cognitive impairment; AD, Alzheimer's disease; VEGF, vascular endothelial growth factor.

Relationships Between CSF NG and Other Variables in the Pooled Sample

As shown in Figure 3, CSF NG was associated with age ($r = -0.14$, $p = 0.02$) and MMSE scores ($r = -0.19$, $p = 0.001$) in the pooled sample. Compared with males, females had significant higher levels of NG ($t = -3$, $p = 0.003$). A β ⁺ individuals had higher levels of NG than A β ⁻ individuals ($t = -7$, $p < 0.001$).

Correlations Between CSF VEGF and NG in Each Diagnostic Group

In the pooled sample, levels of VEGF in CSF were positively associated with CSF NG ($r = 0.23$, $p < 0.001$). In the diagnosis-stratified analyses, we found that VEGF was associated with NG (NC: $r = 0.335$, $p = 0.002$; MCI: $r = 0.257$, $p = 0.037$; AD: $r = 0.259$, $p = 0.037$; Figure 4).

Association with CSF VEGF and NG

To examine the association between CSF VEGF and NG, several linear regression models were conducted (Table 2). In the first model, CSF VEGF levels were significantly associated with NG levels (unstandardized $\beta = 538.9$ (135); $p < 0.001$) without adjusting for other variables. In the second model, a significant association of CSF VEGF with NG was observed after controlling for age,

Table 1 Demographic and Clinical Variables

Clinical variables	NC (n = 83)	MCI (n = 143)	AD (n = 65)	p
Age, y	75.7 (5.6)	74.7 (7.3)	75.4 (7.4)	0.57
Female/male, n	43/40	47/96 ^a	28/37	0.018
Education, y	15.7 (2.96)	15.9 (2.94)	15.2 (2.98)	0.3
APOE4 (+)/(-), n	20/63	77/66 ^a	46/19 ^{b, c}	<0.001
MMSE	29.1 (0.9)	26.9 (1.8) ^a	23.5 (1.89) ^{b, c}	<0.001
CSF, pg/mL				
VEGF (natural log)	2.73 (0.1)	2.71 (0.13)	2.68 (0.11)	0.06
NG	362 (206)	506 (304) ^a	560 (316) ^{b, c}	<0.001
A β 42	206 (53.6)	160 (49.6) ^a	142 (35.7) ^{b, c}	<0.001

Notes: Comparison between NC group and MCI group is marked behind "MCI group", ^a $p < 0.05$; Comparison between MCI group and AD group is marked behind "AD group", ^b $p < 0.05$; Comparison between NC group and AD group is marked behind "AD group", ^c $p < 0.05$.

Abbreviations: NC, normal controls; MCI, mild cognitive impairment; AD, Alzheimer's disease; VEGF, vascular endothelial growth factor; MMSE, mini-mental state examination; NG, neurogranin; A β 42, β -amyloid 42.

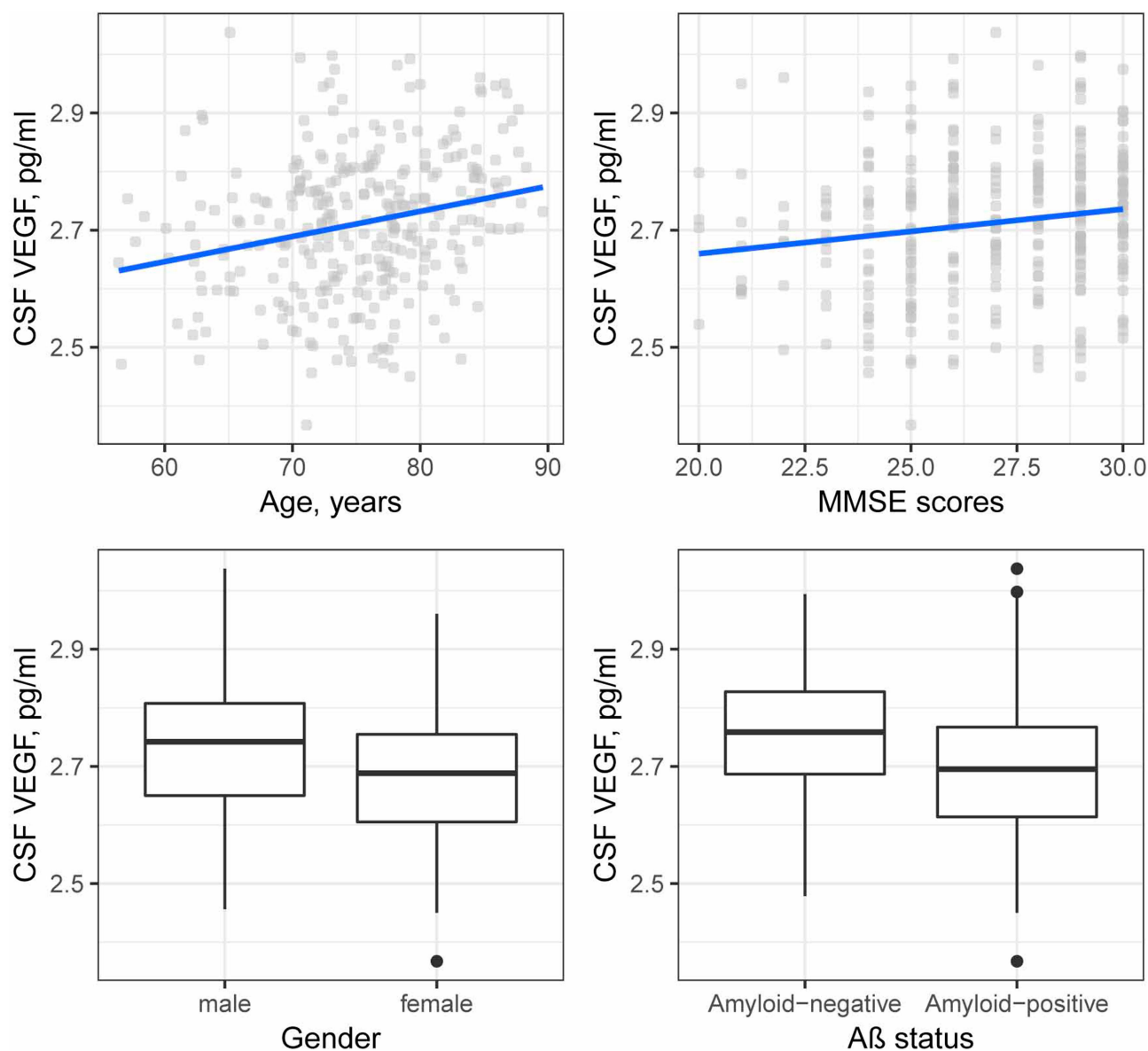


Figure 2 Relationships between VEGF and age, MMSE scores, gender and A β status in the pooled sample.
Abbreviations: VEGF, vascular endothelial growth factor; MMSE, mini-mental state examination; A β , β -amyloid.

gender, educational attainment, APOE4 genotype and clinical diagnosis (unstandardized $\beta = 862$ (126); $p < 0.001$). Finally, in the third model, the association of CSF VEGF with NG was still present after adjusting for A β status (unstandardized $\beta = 948$ (124); $p < 0.001$).

Discussion

To the best of our knowledge, this is the first study to report that CSF VEGF levels were positively associated with CSF NG (reflecting synaptic degeneration) independent of AD pathology among older individuals with different severities of cognitive impairment.

Regarding the biology of VEGF, it is abundantly expressed in the brain and plays important roles in angiogenesis, blood production and neural development.¹⁸ It has also been implicated as a beneficial factor in AD pathogenesis.¹⁹ For instance, patients with AD had lower serum VEGF levels²⁰ and lower cerebral capillary VEGF expression in hippocampus and other AD-related brain regions²¹ compared with control subjects. In addition, previous longitudinal studies investigating the association of CSF VEGF levels with brain aging outcomes found that higher VEGF levels were associated with reduced hippocampal atrophy and reduced decline of cognition over

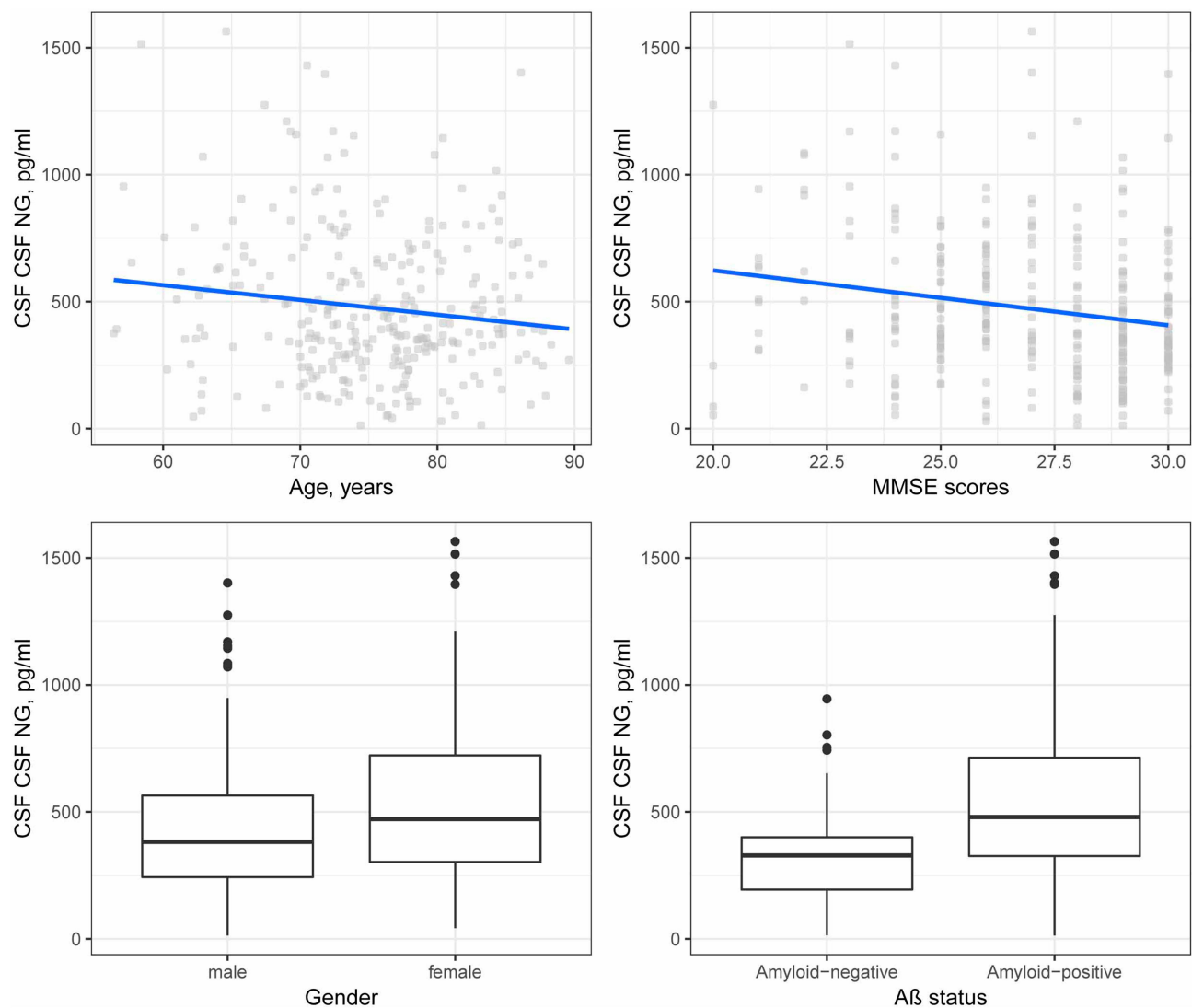


Figure 3 Relationships between NG and age, MMSE scores, gender and A β status in the pooled sample.
Abbreviations: NG, neurogranin; MMSE, mini-mental state examination; A β , β -amyloid.

time.^{12,13} Furthermore, preclinical studies found that the transplantation of nanospheres releasing VEGF or stem cells overexpressing VEGF to AD mice decreases A β accumulation and ameliorates cognitive deficits,^{10,11} suggesting that VEGF treatment may be neuroprotective in AD.¹²

Regarding the biology of neurogranin, it is a postsynaptic protein implicated in long-term potentiation (LTP) and cognition.^{2,22} Neurogranin is primarily expressed in the hippocampus and cortex,^{23,24} which are the same cerebral regions that are affected in patients with AD. It has been reported that neurogranin levels are substantially lower in the cortex and hippocampus of AD patients compared to control individuals.^{24,25} Additionally, Thorsell and his colleagues reported a significant increase of CSF neurogranin levels in patients with AD compared to controls.⁴ One possible

explanation is that dying neurons may lead to the increased neurogranin levels in CSF in patients of AD. Furthermore, greater levels of neurogranin in CSF predicted faster rates of cognitive decline and hippocampal atrophy and progression from MCI to AD.^{2,26} More importantly, Casaletto and colleagues found that neurogranin, but not amyloid or tau pathology, was associated with memory performance in cognitively normal older people, suggesting that neurogranin is highly sensitive to subtle memory abilities and that synaptic dysfunction may precede early AD pathogenesis.²⁷

Regarding VEGF and synaptic function, a previous animal study using mice model of chronic cerebral hypoperfusion (CCH) has shown that VEGF could ameliorate impaired hippocampal synaptic function, including LTP, basal synaptic transmission, depotentiation and paired-

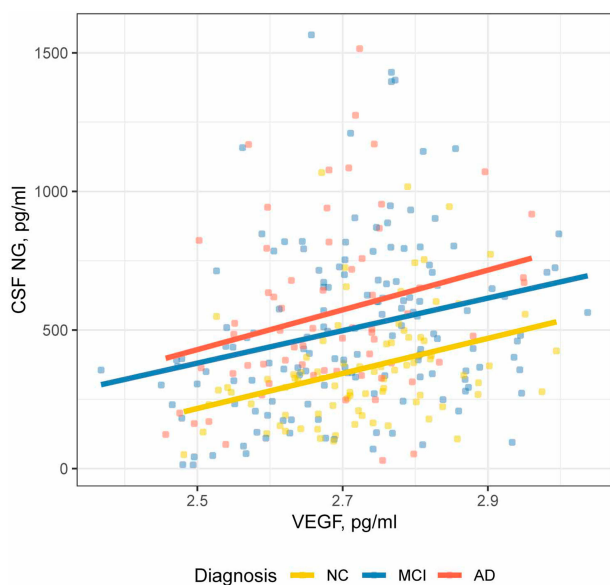


Figure 4 Associations of CSF VEGF with NG levels among the three diagnostic groups. In the diagnosis-stratified analyses, we found that VEGF was associated with NG (NC: $r = 0.335$, $p = 0.002$; MCI: $r = 0.257$, $p = 0.037$; $r = 0.259$, $p = 0.037$). **Abbreviations:** NC, normal controls; MCI, mild cognitive impairment; AD, Alzheimer's disease; VEGF, vascular endothelial growth factor; NG, neurogranin.

pulse facilitation.²⁸ In addition, De Rossi et al found that both VEGF and VEGF receptor 2 (VEGFR2) play important roles in the hippocampal synaptic function and consolidation of fear-related memory.²⁹ It has been reported that in cultured hippocampal neurons, VEGF induces the elevation of protein synthesis, partially by regulating the levels of mammalian target of rapamycin (mTOR) and calcium/calmodulin protein kinase II (CaMKII), indicating that it may contribute to protracted alterations of synaptic efficacy.³⁰ Taken together, these findings highlighted a potentially beneficial role of VEGF in the synaptic structure and function.

In the present study, we found that CSF VEGF levels were positively associated with NG in subjects with different severities of cognitive impairment. One possible explanation for this finding is that VEGF upregulation acts as a compensatory mechanism to reduce synaptic loss caused by AD. It has been reported that cerebral hypoperfusion has been considered as a key feature in AD brain.³¹ The long-lasting hypoperfusion could contribute to both upregulation of VEGF and compromise of neuronal and synaptic survival. Thus, VEGF elevations may play an important role in counteracting the detrimental effects of the AD pathological cascade on synaptic structure and function. However, further preclinical and clinical studies are needed to clarify the mechanisms by which VEGF affects synaptic function in AD.

In the present study, we did not observe a significant difference in CSF VEGF between APOE4 carriers and noncarriers in the whole sample (NC, MCI and mild AD). However, a previously published study³² showed that in AD patients, the severity of dementia was positively associated serum VEGF levels in APOE4 carriers, but not in APOE4 non-carriers. This inconsistency may be due to several differences between studies, such as the sample (CSF vs serum) and participants with different severities of cognitive impairment.

Several limitations should be noted. First, the cross-sectional design does not allow us to explore the temporal relationship between VEGF and NG. The prospective longitudinal studies are needed to examine the association of baseline CSF VEGF with change in CSF NG over time. Second, the ADNI cohort represents a convenience sample of volunteers, which may lead to selection bias. Therefore,

Table 2 Modeling of Potential Association of VEGF with NG

	Model					
	1	P	2	P	3	P
	Beta (se)		Beta (se)		Beta (se)	
VEGF	538.9 (135)	<0.001	862(126)	<0.001	948 (124)	< 0.001
Age, y			-7(2.2)	0.002	-7.4 (2.2)	< 0.001
Gender			156.9(31.7)	<0.001	159 (31)	< 0.001
Education, y			1.2(5.1)	0.8	0.9 (5)	0.85
APOE4 (+) vs (-)			129.3(31)	<0.001	67 (34)	0.048
MCI vs control			141.4(36)	<0.001	95 (37)	0.01
AD vs control			190(44.5)	<0.001	131 (45)	0.004
Aβ+ status					167 (39)	< 0.001

Notes: Linear regression models were applied to investigate the association of CSF NG levels with other variables. Model 1 was unadjusted; model 2 was adjusted for age, gender, education, APOE4 status and diagnosis; and model 3 was additionally adjusted for Aβ42. Beta is unstandardized beta.

Abbreviations: VEGF, vascular endothelial growth factor; NG, neurogranin; MCI, mild cognitive impairment; AD, Alzheimer's disease; Aβ42, β-amyloid 42; T-tau, total tau; se, standard error.

our findings need to be replicated in other samples, particularly in a population-based sample.

In summary, CSF VEGF levels were associated with NG independent of AD pathology and disease stage.

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Disclosure

The authors declare that they have no conflicts of interest.

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