VGF in Cerebrospinal Fluid Combined With Conventional Biomarkers Enhances Prediction of Conversion From MCI to AD

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Background: Previous work has suggested that the brain and cerebrospinal fluid (CSF) levels of a neural protein involved in synaptic transmission, VGF (a noninitialism), may be altered in mild cognitive impairment (MCI) and Alzheimer Disease (AD). The objective of the current work is to examine the potential of CSF levels of a peptide derived from VGF to predict conversion from MCI to AD.

Materials and Methods: Using multivariate analytical approaches, the performance of the conventional biomarkers (CSF Aβ1-42 and phosphorylated tau +/− hippocampal volume) was compared with the same biomarkers combined with CSF VGF peptide levels in a large publicly available data set from human subjects.

Results: It was observed that VGF peptides are lowered in CSF of patients with AD compared with controls and that combinations of CSF Aβ1-42 and phosphorylated tau, hippocampal volume, and VGF peptide levels outperformed conventional biomarkers alone (hazard ratio = 2.2 vs. 3.9), for predicting MCI to AD conversion.

Conclusions: CSF VGF enhances the ability of conventional biomarkers to predict MCI to AD conversion. Future work will be needed to determine the specificity of VGF for AD versus other neurodegenerative diseases.

Key Words: mild cognitive impairment, Alzheimer disease, cerebrospinal fluid, biomarker, VGF, amyloid, tau, hippocampal volume

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Alzheimer disease (AD) is characterized by a prodromal course during which amyloid beta (Aβ) and phosphorylated tau (pTau) deposit into the brain, atrophy is seen in the hippocampus, and disruptions of brain metabolism occur. These pathologic changes have formed the basis for the use of a series of fluid and imaging biomarkers that may be used to (1) achieve earlier diagnoses for patients, (2) predict which individuals are most likely to clinically worsen over time, (3) help to identify and stratify subjects enrolling in AD-related clinical trials, and (4) serve as outcome measurements in AD-related clinical trials.

Recently, our group and others have identified a group of novel plasma and cerebrospinal fluid (CSF) biomarkers that fall outside the traditional Aβ cascade. For example, we used a hypothesis-free bioinformatics approach to identify a panel of 16 peptides in CSF initially identified as showing high diagnostic accuracy for AD versus control, that was highly predictive of conversion from mild cognitive impairment (MCI) to AD in an independent group of subjects and outperformed conventional CSF markers such as Aβ, tau derivatives, and their ratios.[1] These studies highlight noncanonical pathologic cascades that may provide useful tools for clinical practice and clinical trials purposes, and may also reveal new insights about AD disease mechanisms. One of the peptides identified using this hypothesis-free approach to separate AD from normal (NL) controls was VGF.[1] VGF (a noninitialism) has recently received significant attention because of its role in learning and memory and its potential role in the pathophysiology of AD.[2,3] VGF is a neurotrophin-inducible 615-amino acid polypeptide secreted by neurons and is cleaved into multiple smaller fragments ranging in length from 16 to 129 amino acids. VGF is produced in a number of brain regions, including the cerebral cortex, amygdala,

hippocampus, and hypothalamus, and in neuroendocrine tissues such as the adrenal medulla and adenohypophysis, and is thought to be involved in synaptogenesis and energy homeostasis.[4,5] A 2011 study used capillary electrophoresis coupled mass spectrometry and identified peptide fragments of VGF that were lowered in AD patients, and in conjunction with other synaptic peptide fragments, predicted MCI to AD conversion.[6] We and others have also observed altered levels of VGF in the CSF of AD patients compared with controls.[1,7–10] VGF overexpression also protects against memory impairment in 5xFAD transgenic mice that model AD.[2] However, previous work has not yet examined the potential for VGF in the CSF, when combined with established biomarkers, to predict MCI to AD conversion.

Therefore, in the current study we examined the potential for CSF VGF, when combined with conventional biomarkers of CSF Aβ1-42, total tau (tTau) and pTau-181, and hippocampal volume (HV), to enhance the diagnostic and prognostic accuracy of these markers. The focus of this work is on the VGF peptide fragment with sequence NSEPQDEGELFQGVDPR (VGF.NSEP) because it previously emerged as a strong predictor in a panel of peptides that predict MCI to AD conversion,[1] though other VGF peptide fragments are also examined. Unlike our previous studies involving hypothesis-free approaches to identify optimal peptides to include in biomarker signatures,[1,11] the current study was focused specifically on the utility of VGF using data from 2 independent groups in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort: one group of AD and NL subjects and a separate group of MCI subjects.

MATERIALS AND METHODS

The data used for this research are identical to those used in Devanarayan et al.[11] The ADNI database (adni.loni.usc.edu) utilized in this research was launched in 2003 as a public-private partnership, led by the principal investigator, Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), PET, other biological markers, and clinical and neuropsychological assessments can be combined to measure the progression of MCI and early AD. For up-to-date information, see www.adni-info.org. This study was conducted across multiple clinical sites and was approved by the Institutional Review Boards of all of the participating institutions. Informed written consent was obtained from all participants at each site. Data used for the analyses presented here were accessed on February 24, 2018. Although the ADNI database continues to be updated on an ongoing basis, most newly added biomarker data are from later time points (ie, beyond 1 y), in contrast to the baseline data used in this study.

Subjects

This research was focused on the relationship between VGF, conventional biomarkers (CSF amyloid/tau and MRI HV) and therefore, only those subjects whose values for these markers were available at baseline were included. Ultimately, this data set included 287 subjects across the 3 diagnostic categories (AD, MCI, and NL). NL subjects were defined as those without memory complaints and a clinical dementia rating (CDR) score of 0. MCI subjects had CDR scores of 0.5, had an abnormal score on Wechsler Memory Scale Revised-Logical Memory II and did not have significant functional impairment. AD subjects had functional decline and CDR score of 0.5 or 1.0.

Hippocampal Volume

HV was chosen given its ability to predict MCI to AD conversion[12] and its incorporation into proposed schema to classify AD subjects.[13] HV was obtained from MRI scans (mostly 1.5 T; 25% in this data set had 3.0 T scans) and was computed using FreeSurfer. Please see "UCSF FreeSurfer Methods" PDF document under "MR Image Analysis" in the ADNI section of https://ida.loni.usc.edu/ for details.

CSF Samples

Innogenetics' INNO-BIA AlzBio3 immunoassay on a Luminex xMAP platform was used to measure levels of the conventional biomarkers Aβ1-42, tTau, and pTau-181 in CSF. The Caprion Proteomics mass spectrometry platform was used to measure levels of individual peptides. The VGF peptides (sequence NSEPQDEGELFQGVDPR, referred to here as VGF.NSEP, sequence AYQGVAAPFPK, referred to here as VGF.AYQG, and sequence THLGEALEPLSK, referred to here as VGF.THLG) used in this study were among 320 peptides generated from tryptic digests of 143 proteins. The details regarding the measurements of these peptides can be found in the Use of Targeted Mass Spectrometry Proteomic Strategies to Identify CSF-based Biomarkers in Alzheimer's Disease Data Primer (found under Biomarkers Consortium CSF Proteomics multiple reaction monitoring Data Primer at ida.loni.usc.edu) and in the paper by Spellman et al.[14]

Statistical Methods

As we have described previously,[11] optimal combinatorial signatures including CSF Aβ1-42, tTau, pTau-181, their ratios, HV and VGF-derived peptides with simple decision thresholds for each marker were first identified from the AD and NL subjects. These signatures were revealed by an unbiased, data-driven manner via regression and tree-based computational algorithms called Patient Rule Induction Method[15] and Sequential BATTing.[16] To measure the performance of each signature for disease-state differentiation (ie, NL vs. AD), 5-fold cross-validation was performed. To do this, the data were randomly divided into 5 subgroups, referred to as folds, and a signature was derived from the remaining 4 folds. This signature was then tested on the left-out fold. This process was iterated 10 times and a median value of the performance measures, positive predictive value (PPV), negative predictive value (NPV) and accuracy was calculated.

The optimal signature for differentiating NL and AD subjects was then assessed to determine whether it can also predict which MCI subjects at baseline would convert to AD in the future. Baseline values for Aβ1-42, tTau, pTau-181, HV, and VGF peptides for each MCI subject were used to classify each subject as being "signature positive" (ie, similar to the profile found in AD) or "signature negative" (ie, similar to the profile found in NL). PPV, NPV, and accuracy were then computed by comparing the actual outcome (conversion or not to AD over 36 mo) to the predicted outcome (signature positive/negative which would predict conversion/nonconversion, respectively). Exact McNemar test was used to compare PPV, NPV, and accuracy values between the signatures.

In addition to measuring the performance of accurately predicting whether the MCI subjects would convert to AD over 36 months, Kaplan-Meier analysis of the time to conversion from MCI to AD was carried out using available data up to 10 years after the initial evaluation. Potential markers for this analysis were grouped into the following categories:

(1) Demographic markers (presence of APO-E4 allele, age, sex, education).
(2) Demographic markers+HV.
(3) Demographic markers+amyloid/tau CSF markers (called "AT": Aβ1-42, tTau, pTau-181, ratios of tTau to Aβ1-42, and pTau-181 to Aβ1-42).
(4) Demographic markers+HV+AT.
(5) Demographic markers+HV+AT+VGF.

From this analysis, estimates of the median, 25th and 75th percentiles of the time to progression were derived for the signature positive and signature negative groups. In addition, Cox proportional hazards model was used to estimate the hazard ratio, which reflects the increase in instantaneous risk of the progression from MCI to AD at any given point in time. For example, a hazard ratio of 2 would imply that at any particular time, twice as many MCI subjects in the signature positive group would convert to AD compared with the signature negative group. The validity of proportional hazards assumption of the Cox proportional hazards model was verified by the χ^2 test.

All analyses related to predictive modeling and signature derivation were carried out using R (www.R-project.org), version 3.4.1, with the publicly available package, SubgrpID.[16] The time to progression analysis of the derived signatures and related assessments were carried out using JMP, version 13.2 and the verification of the proportional hazards assumption in the Cox proportional hazards model was carried out using the cox.zph function in the survival package of R.

RESULTS

Demographics

Basic demographic data and data involving conventional biomarkers are identical to the paper by Devanarayan et al.[11] Sixty-six AD, 135 MCI, and 86 NL subjects were included in the analysis. There were no statistically significant differences in terms of age (range of means, 75.1 to 75.8 y, $P > 0.05$) and education (range of means, 15.1 to 16 y, $P > 0.05$). There was a greater number of males than females (59.1% vs. 40.9%), though their likelihood of conversion from MCI to AD over 36 months was similar (43.5% vs. 53.9%, $P = 0.285$, χ^2 test). Formal analysis of biomarkers was not broken down by sex given the relatively small number of female MCI subjects (n = 44) in this data set. The likelihood that an APO-E4 allele was present was higher in AD than in other subjects (present in 71.2% AD, 50% MCI, and 24.4% NL subjects; $P < 0.0001$; χ^2 test) and was a relatively weak risk factor for the conversion of MCI to AD (present in 40/62 converters and 31/70 nonconverters, $P = 0.03$, χ^2 test), both of which have been demonstrated previously.[17,18]

Disease State Classification—Univariate Analysis

Figures 1A–D recapitulate previous analysis[11] showing that Aβ1-42, tTau, pTau-181, and HV are all significantly different between NL and AD subjects. These data are shown again here for ease of comparison to the VGF data ($P < 0.0001$ in all cases) and that these values are intermediate for MCI subjects. However, it should be noted that there is a substantial overlap between the distributions in each diagnostic category, rendering these biomarkers unsuitable for use in isolation for diagnostic categorization. As shown in Figures 1E and F, CSF VGF.NSEP levels are depressed in AD patients compared with NL subjects ($P = 0.0002$) and lower levels at baseline are found in MCI-AD converters than nonconverters ($P = 0.032$).

Disease State Classification—Multivariate Analysis

To determine if combinations of conventional biomarkers +/− the VGF.NSEP peptide are useful in disease-state classification, data-driven algorithms were used to derive the optimal signature that distinguished NL and AD. The performances of these signatures are summarized in Table 1. The signatures are grouped into 6 different categories, as described in the Materials and Methods section, and took relatively simple forms. The best performing signature for disease-state classification was a combination of HV+APO-E4 status, with an accuracy of 79.6%. Adding conventional CSF markers (Aβ1-42, tTau and pTau-181, and their ratios) did not enhance this value (accuracy = 76.3%), nor did the addition of VGF.NSEP peptide (accuracy = 75.7%).

Prediction of the Likelihood of MCI to AD Progression

As described above, for disease state classification, no advantage was found when adding the VGF.NSEP peptide to the conventional markers (overall accuracy of 76.3% vs. 75.7%, $P > 0.05$). However, the combined biomarker signature (HV+AT+VGF) significantly outperformed conventional biomarkers (HV+AT) for the prediction of MCI to AD conversion over 36 months ($P = 0.00013$). Most of the impact of the addition of VGF was in increasing the NPV (from 70.2% to 79.2%, $P < 0.0001$) whereas the impact on PPV was more modest (60.2% to 62.1%, $P = 0.008$). The signature derived from the conventional and novel markers took a simple form, with a cut-point on each of them; HV < 7.81 cm^3, pTau > 16.18 pg/mL, ratio of tTau to Aβ1-42 > 0.29, and VGF.NSEP peptide <20.39 intensity units. Thus, the addition of a novel VGF peptide to the conventional AD markers provides a simple biomarker signature that improves the prediction of 36-month disease progression in MCI subjects at baseline.

Prediction of Time to AD Progression From MCI

Using available information containing 3 to 10 years of follow-up clinical data, the difference in the future time to progression was assessed between the signature positive and signature negative groups from the optimal signatures defined above. Table 2 includes summary measures of the times to progression of the signature negative and signature positive subjects from the Kaplan-Meier analysis and the overall hazard ratios with 95% confidence intervals from the Cox proportional hazards model. The proportionality of hazards assumption from the Cox proportional hazards model used to estimate the hazard ratio was verified by the χ^2 test, and found to be acceptable. All groups containing conventional biomarkers (combinations of CSF amyloid/tau, HV, and APO-E4 status) had similar times to progression (range for second quartile or median, 25.7 to 31.5 mo for signature positive subjects) and hazard ratios (range, 1.9 to 2.2). By comparison, the signature containing VGF.NSEP and conventional markers performed considerably better with median time to progression of 24.1 months and 96.2 months for the signature positive and signature negative groups, respectively, and hazard ratio of 3.9. This difference in hazard ratio is illustrated in Figure 2A

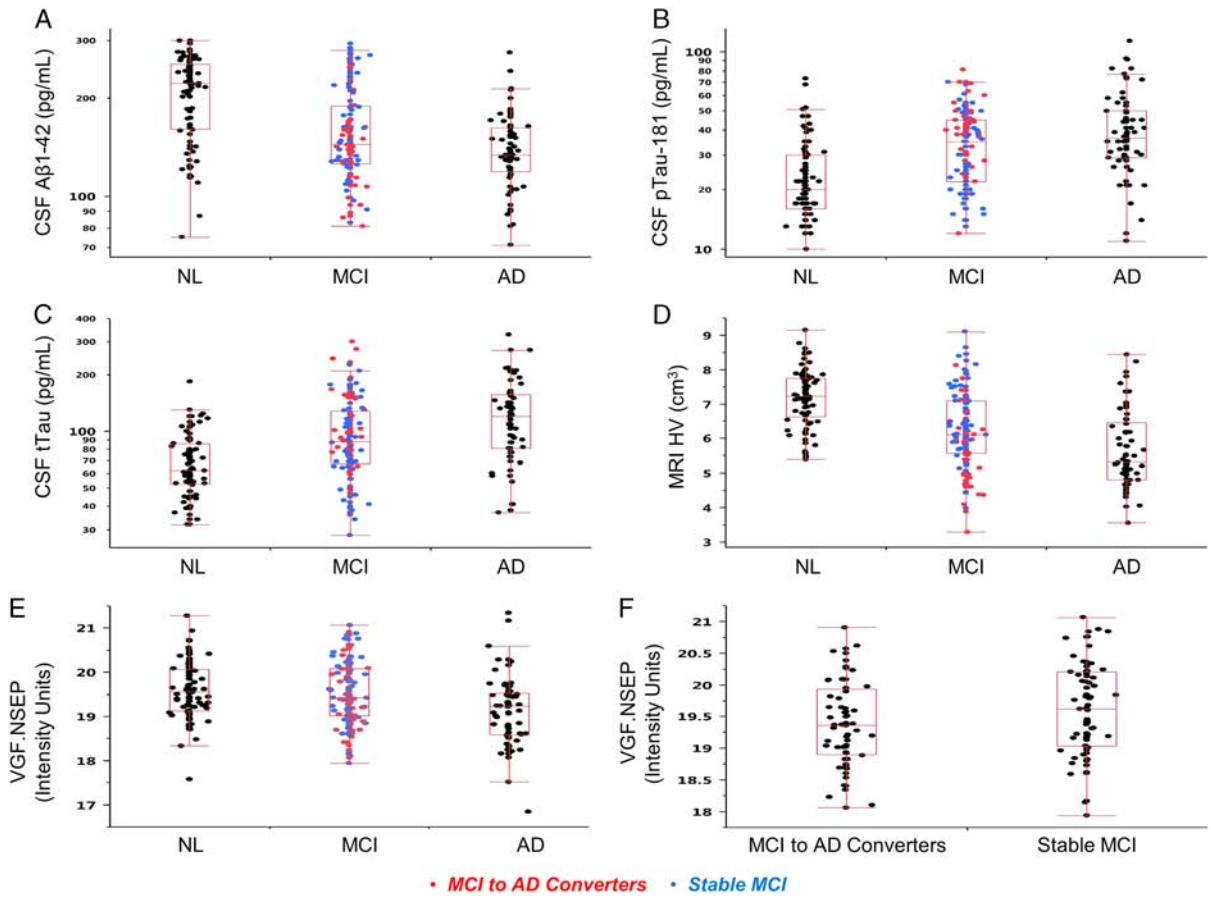

FIGURE 1. Distributions of biomarkers in NL, MCI, and AD subjects: Aβ1-42 (A), pTau-181 (B), tTau (C), HV (D), VGF.NSEP levels (E) (shown in normalized and log2 transformed intensity units), and (F) baseline VGF.NSEP levels in MCI to AD converters and stable MCI subjects over 36 months. In (A)–(E), for the MCI subjects, those that progressed to AD over 36 months are shown in red and those that were stable are shown in blue. The bottom and top ends of the box represent the first and third quartiles, respectively, with the line inside the box representing the median. Lines extending out of the ends of the box indicate the range of the data, minus the outliers. The points outside the lines are the low and high outliers. In (A)–(E), $P < 0.0001$ when comparing NL and AD subjects in (F), $P = 0.032$ when comparing converters to stable MCI. AD indicates Alzheimer disease; CSF, cerebrospinal fluid; HV, hippocampal volume; MCI, mild cognitive impairment; NL, normal.

TABLE 1. Performance Summary of Optimal Signatures

		AD Versus Normal Diagnosis (Internal Cross-validation)			36-Month MCI Progression to AD (Independent Validation)		
Data Types	Diagnostic Criteria for Signature Positive	% PPV	% NPV	% Accuracy	% PPV (MCI to AD)	% NPV (Stable MCI)	% Accuracy
AT	tTau/Ab1-42 > 0.59	71.6	80.5	76.5	58.1	66.1	61.7
HV	HV < 6.41 and ApoE4 +	92.7	74.8	79.6	61.2	60.5	60.7
AT+HV	HV < 7.0, pTau > 18.1, and tTau/Ab1-42 > 0.36	73.4	78.4	76.3	60.2	70.2	64.4
VGF	VGF.NSEP < 19.71 and ApoE4 +	69.1	79.1	70.4	65.9	61.5	63.0
AT+VGF	pTau/Ab1-42 > 0.08, tTau/Ab1-42 > 0.31, and VGF.NSEP < 20.30	75.4	75.8	75.7	59.6	76.1	65.2
AT+HV+VGF	HV < 7.81, pTau > 16.18, tTau/Ab1-42 > 0.29, and VGF.NSEP < 20.39	72.3	78.2	75.7	62.1	79.2	68.1

AD indicates Alzheimer disease; AT, amyloid/tau; HV, hippocampal volume; MCI, mild cognitive impairment; NPV, negative predictive value; PPV, positive predictive value.

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TABLE 2. Time to Progression (T2P) of MCI Subjects to AD Using Optimal Signatures

		Signature Negative		Signature Positive		
Data Types	Diagnostic Criteria for Signature Positive	N	T2P (mo) Q1, Q2, Q3	N	T2P (mo) Q1, Q2, Q3	Hazard Ratio (95% CI)
AT	tTau/Ab1-42 > 0.59	59	23.4, 71.6, 108	76	13.6, 25.7, 72.0	1.9 (1.2, 3.1)
HV	HV < 6.41 and ApoE4+	86	18.6, 48.2, 108	49	13.1, 31.5, 60.0	2.0 (1.3, 3.2)
AT+HV	HV < 7.0, pTau > 18.1, and tTau/Ab1-42 > 0.36	57	24.4, 71.6, 108	78	12.6, 25.7, 72.0	2.2 (1.4, 3.6)
VGF	VGF.NSEP < 19.71 and ApoE4 +	91	24.0, 48.0, 96.5	44	12.2, 18.1, 71.6	2.1 (1.3, 3.2)
AT+VGF	pTau/Ab1-42 > 0.08, tTau/Ab1-42 > 0.31, and VGF.NSEP < 20.30	46	38.8, 96.5, 108	89	12.6, 24.1, 54.9	3.4 (2.1, 5.9)
AT+HV+VGF	HV < 7.81, pTau > 16.18, tTau/Ab1-42 > 0.29, and VGF.NSEP < 20.39	48	38.8, 96.2, 120	87	12.4, 24.1, 60	3.9 (2.3, 7.0)

AD indicates Alzheimer disease; AT, amyloid/tau; CI, confidence interval; HV, hippocampal volume; MCI, mild cognitive impairment.

(without VGF) and Figure 2B (with VGF), where the Kaplan-Meier curves demonstrate time to progression profiles of the signature positive versus signature negative MCI subjects at baseline. The increased separation of the time to progression curves in Figure 2B (with VGF) demonstrates the faster progression experienced by the MCI subjects meeting this signature criterion at baseline.

Studies of VGF Peptide

To determine whether the impact of VGF was isolated to the particular peptide fragment (VGF.NSEP) that emerged from the multivariate analysis in the study by Llano et al,[1] the other 2 VGF peptides (VGF.AYQG and VGF.THLG) in this 320-peptide multiple reaction monitoring panel were also assessed. The pairwise correlations are over 97% between the 3 VGF peptides, and therefore as expected, the other 2 VGF peptides have very similar effects across the disease states (NL vs. AD significant with $P < 0.05$) and differ significantly ($P < 0.05$) between the stable and progressive MCI groups. When replacing the VGF.NSEP peptide which each of these other 2 peptides one at a time, the performance of the combined signature for the HV+AT+VGF scenario was quite similar in terms of the median time to progression of MCI subjects to AD (Table 3). However, the differences were greater in the overall time course of progression that resulted in larger hazard ratios (4.1 and 4.7). Thus, the considerable improvement we see in the prediction of MCI to AD progression by including VGF with the conventional markers is evident for all the 3 peptide fragments of VGF, and not isolated to a specific peptide fragment.

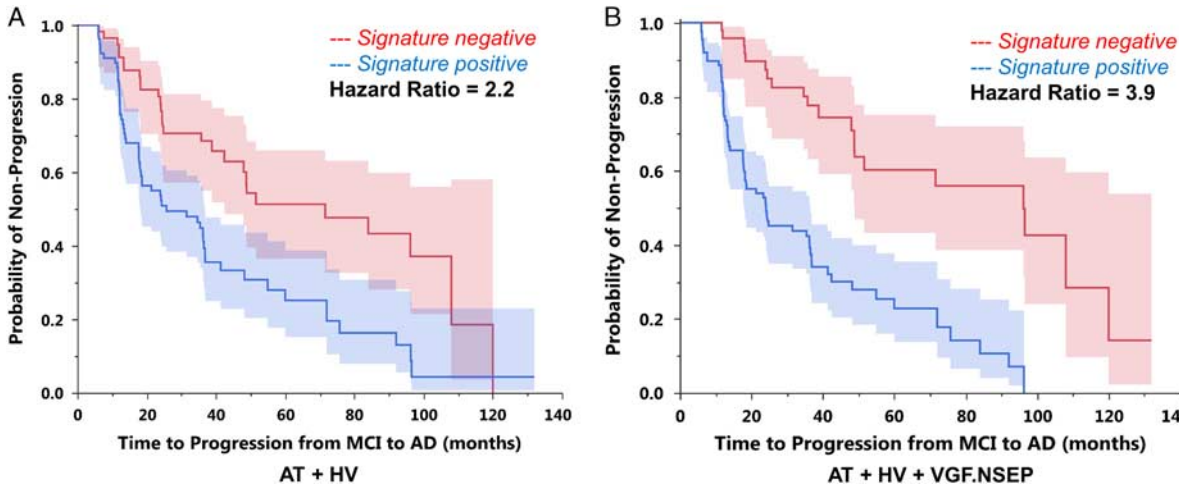

FIGURE 2. Time to progression profiles of the signature positive versus signature negative MCI subjects with the shaded 95% confidence intervals are shown here by Kaplan-Meier analysis. The effect of signature based on only the conventional markers (HV and AT) is illustrated in (A) and the signature with both the conventional markers and the novel VGF.NSEP peptide from the MRM panel is shown in (B). Patients meeting the signature criterion that includes the VGF.NSEP peptide experience 3.9-fold faster progression to AD at any given time (hazard ratio = 3.9), relative to the 2.2-fold faster progression without this peptide. AD indicates Alzheimer disease; AT, amyloid/tau; HV, hippocampal volume; MCI, mild cognitive impairment; MRM, multiple reaction monitoring.

TABLE 3. Time to Progression (T2P) of MCI Subjects to AD Using Each VGF Peptide

Data Types	Diagnostic Criteria for Signature Positive	Signature Negative N	Signature Negative T2P (mo) Q1, Q2, Q3	Signature Positive N	Signature Positive T2P (mo) Q1, Q2, Q3	Hazard Ratio (95% CI)
AT+HV+VGF	HV < 7.81, pTau > 16.18, tTau/Ab1-42 > 0.29, and VGF.NSEP < 20.39	48	38.8, 96.2, 120	87	12.4, 24.1, 60.0	3.9 (2.3, 7.0)
	HV < 7.81, pTau > 16.18, tTau/Ab1-42 > 0.29, and VGF.AYQG < 18.47	56	35.8, 96.2, 120	79	12.3, 23.4, 48.3	4.1 (2.4, 6.8)
	HV < 7.81, pTau > 16.18, tTau/Ab1-42 > 0.29, and VGF.THLG < 17.62	52	48.0, 96.5, 120	83	12.3, 23.9, 48.3	4.7 (2.7, 8.2)

AD indicates Alzheimer disease; AT, amyloid/tau; CI, confidence interval; HV, hippocampal volume; MCI, mild cognitive impairment.

DISCUSSION

Summary

We examined the ability of CSF VGF-derived peptides, in combination with conventional AD biomarkers (Aβ1-42, tTau, pTau-181, their ratios, and HV) to serve as a disease-state marker, and to predict conversion from MCI to AD in a separate group of subjects. We observed that CSF levels of a VGF peptide, on its own, are lower in AD subjects than NLs and that lower levels predict MCI to AD conversion. When combined with conventional biomarkers, the VGF peptide significantly increased the ability of a combination of conventional biomarkers to predict MCI to AD conversion, with the hazard ratio increasing from 2.2 to 3.9. These data suggest that VGF may play a previously underrecognized role in the pathophysiology of AD and that CSF VGF may be useful to help predict MCI to AD conversion.

Total Tau Versus Phosphorylated Tau in Predicting MCI to AD Conversion

It is notable that, when combined with HV, Aβ1-42 and VGF.NSEP, CSF was found to play an important role along with pTau for the prediction of MCI to AD progression. tTau, but not pTau-181, elevations in the CSF have been observed in many non-AD neurological conditions,[19–21] suggesting that tTau is a general marker of neuronal injury, whereas pTau-181 better reflects AD pathology. The finding in the current study that tTau plays an important role along with pTau for the prediction of MCI-AD conversion is aligned with the previous data showing that tTau is more predictive than pTau-181 in predicting subsequent cognitive decline in MCI and AD.[22,23] These findings suggest that although pTau-181 may be more useful as a disease-state marker, particularly when making a differential diagnosis, tTau is also an important marker of disease activity and thus the current rate of clinical decline. In addition, because the database we used only captures the progression to AD of these MCI subjects, and not the other neurodegenerative diseases, it is likely that the use of pTau-181 instead of tTau in our signature may have shown improved performance specificity if we had applied it to a broader group of MCI subjects that also progressed to other forms of dementia.

VGF and AD

The current finding that all peptides associated with VGF are diminished in the CSF of AD patients compared with controls is consistent with the previous studies comparing VGF peptide or protein levels in CSF[6,8,10] and brain tissue (parietal cortex[3]) from AD and control subjects. The functional significance of this decrease is not yet clear, but may relate to VGF's potential role in synaptic plasticity and/or neuronal metabolism. VGF is found widely throughout the brain, including areas highly affected in AD such as cerebral cortex, hippocampus, entorhinal cortex, basal forebrain, amygdala, and brainstem.[3,24,25] Its expression is upregulated by neuronal activity[26] and can be induced by neuronal growth factors such as brain-derived neurotrophic factor.[24,27] In animal models, VGF has been shown to be important for the mediation of synaptic plasticity and neurogenesis.[28,29] Knockout of this gene has been shown to cause diminished body weight and percent body fat,[30] whereas overexpression may protect the brain against AD-related pathology.[2] These functions may align with the loss of hippocampal function and loss of body weight and percent body fat seen in AD.[31,32]

The mechanism behind the drop in VGF levels in AD CSF is not yet clear. Given the parallel drop in the cerebral cortex,[3] low levels in the CSF are likely not due to a shift of VGF from CSF to parenchyma, as has been hypothesized for Aβ in the CSF of AD patients. Low levels of VGF in CSF (and brain) may suggest that VGF is a general marker for neuronal loss, consistent with the drop in CSF VGF in frontotemporal dementia,[33] potentially putting VGF into the "neurodegenerative/neuronal injury" class of biomarkers in the AT(N) framework previously described.[34] Future work examining VGF across other states of neuronal injury may help to add clarity to this issue. One previous study observed borderline elevations of VGF in the CSF of MCI compared with control and AD subjects, and that VGF elevations in MCI subjects predicted later conversion to AD.[10] Such transient elevations are reminiscent of "pseudonormalization" of other biomarkers whose values in MCI appear to change in the opposite direction than that seen in AD.[1,35,36]

Implications of the Prediction of MCI-AD Conversion

CSF Aβ1-42 and tau derivatives as biomarkers are well-established for the prediction of clinical decline in MCI and the predictive accuracy of these markers increases when they are combined with volumetric imaging.[37,38] Both of these findings were reproduced in the current study (Table 1). In addition, recently a number of non-Aβ, non-tau CSF markers have been found that separate AD from NL subjects, and these

markers have been implicated across a number of metabolic, inflammatory, and synaptic physiology pathways.[6,7,9] A small number have also shown the ability to predict MCI to AD conversion. For example, heart fatty acid binding protein, chemokine receptor 2, neurogranin, calbindin, IL-1, and thymus-expressed chemokine have all individually been shown to predict MCI to AD progression.[1,14,39–42] In addition, we and others identified panels of peptides that predict MCI to AD progression.[1,14] These data point to a range of potential pathophysiological mechanisms implicated in AD outside of the classical amyloid-driven cascade. In addition, like most of the previous work, the current study did not examine non-AD dementia or other neurologic disease. Therefore, it will be important in future studies to include non-AD dementias and other neurological illness to determine the specificity of VGF and other molecules as biomarkers for AD and predictors of MCI to AD progression.

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