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RESEARCH ARTICLE

Plasma EGF and cognitive decline in Parkinson's disease and Alzheimer's disease

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Abstract

Objective: Cognitive decline occurs in multiple neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease (PD). Shared underlying mechanisms may exist and manifest as shared biomarker signatures. Previously, we nominated plasma epidermal growth factor (EGF) as a biomarker predicting cognitive decline in patients with established PD. Here, we investigate EGF as a predictive biomarker in prodromal PD, as well as AD. **Methods**: A cohort of PD patients (n = 236) was recruited to replicate our finding that low baseline EGF levels predict future cognitive decline. Additionally, plasma EGF and cognitive outcome measures were obtained from individuals with normal cognition (NC, n = 58), amnestic mild cognitive impairment (AD-MCI, n = 396), and Alzheimer's disease (AD, n = 112) in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort to investigate whether low EGF levels correlate with cognitive status and outcome in AD-MCI and AD. Third, plasma EGF and cognitive measures were evaluated in the high-risk asymptomatic Parkinson's Associated Risk Study (PARS) cohort (n = 165) to investigate the association of EGF and cognitive performance in a PD prodromal context. **Results**: In both PD and AD-MCI, low baseline plasma EGF predicted poorer long-term cognitive outcomes. In asymptomatic individuals at highest risk for developing PD from the PARS cohort, low baseline plasma EGF associated with poorer performance in the visuospatial domain but not in other cognitive domains. **Interpretation**: Low plasma EGF at baseline predicts cognitive decline in both AD and PD. Evidence for this signal may exist in prodromal stages of both diseases.

Introduction

Cognitive decline is a prominent feature of multiple neurodegenerative diseases. In Alzheimer's disease (AD), cognitive decline with progression to dementia is the defining clinical feature, accompanied by development of the neuropathological hallmarks of beta-amyloid plaques and tau-containing neurofibrillary tangles.[1] In Parkinson's disease (PD), cognitive decline is a secondary feature that nevertheless affects the vast majority of PD patients.[2]

Multiple lines of evidence suggest that common mechanisms may contribute to cognitive decline in both PD and AD. Neuropathologically, although PD is defined by alpha-synuclein-containing Lewy body pathology,[3] many PD patients are found at autopsy to have coexisting AD pathology.[4] Some PD patients have been reported to demonstrate beta-amyloid deposition in life by radiotracer imaging as well.[5] Biochemically, in vitro evidence suggests that alpha-synuclein and tau can synergize in the formation of the amyloid fibrils[6] that may initiate pathophysiology. Genetically, mechanisms involving tau – which has been extensively studied in AD[7] – are also implicated by genome-wide association with risk of developing PD.[8] Finally, from the biomarker literature, lower Cerebrospinal fluid (CSF) levels of beta-amyloid, in addition to providing diagnostic support in AD,[9] may predict cognitive decline over time in PD.[10]

We previously screened 102 plasma proteins in an effort to discover blood-based biomarkers predicting cognitive performance in PD. From this screen, we nominated epidermal growth factor (EGF) as a candidate biomarker: low plasma EGF levels associated cross-sectionally with poorer cognition in PD and predicted, in a small cohort of 49 non-demented PD patients followed longitudinally, a substantially increased risk of progression to dementia.[11] Both our cross-sectional and predictive associations were subsequently independently replicated in early, drug-naïve PD subjects.[12]

As a trophic factor, EGF as a candidate biomarker for neurodegenerative diseases such as PD is biologically attractive. Delivery of trophic factors to the brain to ameliorate neurodegeneration has shown efficacy in animal models of PD and AD.[13,14] Indeed, such an approach is currently being tested in human trials.[15] Thus, one may hypothesize that individuals with lower levels of trophic factor support may be at risk of accelerated neurodegenerative disease progression. In the case of EGF, in particular, dopamine-triggered adult neurogenesis in the subventricular zone has been reported to depend on EGF and its cognate receptor, EGFR,[16,17] and local administration of EGF in an animal model of PD has been reported to rescue neurodegeneration.[18]

Because many of these potential mechanisms may extend beyond PD-related neurodegeneration, we sought to understand whether plasma EGF levels predict disease course in PD as well as AD. Furthermore, because increasing evidence suggests that pathophysiology is well underway years or decades before clinical manifestation, we investigated EGF levels in both manifest and prodromal stages of PD and AD. Finally, given the well-known failures of many candidate biomarkers to replicate, we sought to replicate our previous finding in a large cohort of PD patients followed longitudinally over many years.

Materials and Methods

Clinical cohorts

Three clinical cohorts were used in this study (Table 1). In each case, all available subjects meeting the stated criteria were included, without additional selection for subjects with particular EGF values, cognitive profiles, or other characteristics.

UPenn Cohort

Two hundred and thirty-six patients with a diagnosis of idiopathic PD based on UK PD Society Brain Bank clinical diagnostic criteria[19] were recruited to the University of Pennsylvania (UPenn) for this study. None of these individuals were from the 49-person cohort in which we

Table 1. Overview of the three major cohorts with plasma samples included in this study.

Cohort	UPenn	ADNI	PARS
Number	236	566	165
Disease	PD	NC (n = 58), AD-MCI (n = 396), AD (n = 112)	Asymptomatic PD
Age	66.5 ± 9.6	74.7 ± 7.3	65.6 ± 8.2
Sex (% Male)	58.6	62.0	73.1
UPDRS-III	23.3 ± 12.3	–	–
Cognitive Test	MoCA	MMSE	Multiple cognitive tests
EGF Assay	ELISA	Multiplex Immunoassay	ELISA

Means ±/− Standard deviations are shown.

The PARS study includes 29 cognitive tests; see Methods and Data S1 for details on how cognitive test results were standardized and grouped into cognitive domains.

UPDRS-III, motor section of the Unified Parkinson's Disease Rating Scale; ADNI, Alzheimer's Disease Neuroimaging Initiative; PARS, Parkinson's Associated Risk Study; MoCA, Montreal Cognitive Assessment; MMSE, Mini Mental Status Exam; NC, neurologically normal control; AD-MCI, amnestic mild cognitive impairment; AD, Alzheimer's Disease.

first reported our finding that low plasma EGF levels predicted future cognitive decline.[11] These 236 patients represent all of the available PD subjects with (1) plasma samples, and (2) Montreal Cognitive Assessment (MoCA) data available who (3) were not previously reported. They were recruited by the Clinical Core of the UPenn Udall Center. Whole blood samples were obtained and processed for plasma as previously described.[11]

ADNI Cohort

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2004 as a public–private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD).

Data from 566 individuals consisting of normal controls (NC, $n = 58$), individuals with amnestic mild cognitive impairment (AD-MCI, $n = 396$), and individuals with Alzheimer's disease (AD, $n = 112$) from ADNI[20] were obtained (Table 2). In these individuals, cognition was assessed by the Mini-Mental State Examination (MMSE), and plasma EGF levels were measured by multiplex immunoassay by ADNI investigators, as previously described.[21] We included all ADNI research participants for which cognitive class, baseline plasma EGF values, and follow-up were available.

PARS Cohort

One hundred and sixty-five at-risk, asymptomatic individuals enrolled in the Parkinson's Associated Risk Study (PARS) formed the final cohort. PARS participant recruitment and exclusion/inclusion criteria have been described previously.[22,23] At the time of EGF measurement, 198 PARS subjects had been enrolled, of which 181 had completed a comprehensive neuropsychological test battery (see Table S1). Plasma samples were obtained from these individuals, as previously described.[11] For 165/181 (91%) of these PARS subjects with neuropsychological test data, EGF measures passed QC (coefficients of variation [CV] < 0.2), and these 165 individuals were included in this study.

Table 2. Subgroup details for ADNI subjects included in this study.

ADNI Cohort	NC	AD-MCI	AD
Number	58	396	112
Age	75.1 ± 5.8	74.7 ± 7.4	74.8 ± 8.0
Sex (% Male)	51.7	64.6	58.0
MMSE	28.9 ± 1.2	27.0 ± 1.8	23.6 ± 1.9

Means ± Standard deviations are shown.
ADNI, Alzheimer's Disease Neuroimaging Initiative, MMSE, Mini Mental Status Exam; NC, neurologically normal control; AD-MCI, amnestic mild cognitive impairment; AD, Alzheimer's Disease.

EGF Quantification

Plasma EGF levels were measured in the UPenn and PARS cohorts by enzyme-linked immunosorbent assay (ELISA) as previously described.[11] For each cohort, ELISA runs were performed in one batch, on a single day, using a shared set of standards for relative quantification. Samples with CV less than 0.2 across technical duplicates were included in final analyses; greater than 90% of samples passed QC. For the ADNI cohort, plasma EGF levels were measured by multiplex immunoassay (Rules-Based Medicine), as described previously.[24] Methods of EGF quantification differed by cohort because in the ADNI cohort, EGF levels were measured as part of a larger biomarker study assessing ~100 plasma proteins.[24] Because the multiplex immunoassay method has not been demonstrated to be reliable for absolute quantification, all ADNI samples were run in one batch to allow for reliable relative quantification.

EGF quantification was performed blinded to the cognitive status of subjects.

Cognitive and motor testing

For the UPenn cohort, cognitive status was evaluated with the Montreal Cognitive Assessment (MoCA)[25] at baseline and on annual follow-up visits. Motor severity was evaluated using the motor portion of the Unified Parkinson's Disease Rating Scale (UPDRS-III)[26] at baseline and on annual follow-up visits. For the ADNI cohort, cognitive status was evaluated with the MMSE at baseline and on follow-up visits as previously described,[20] and all MMSE scores greater than or equal to 10 were used (28/2472, or 1%, of the MMSE scores obtained from the ADNI database were removed as outliers comprising very low, zero, and negative numbers). For the PARS cohort, cognition was evaluated using a neuropsychological test battery spanning five cognitive domains: verbal memory, executive function/working memory, processing speed/attention, visuospatial function, and language. Because multiple individual cognitive tests may be measuring the same underlying phenomenon, individual tests were categorized and summarized into these five cognitive domains, as previously described.[22] In brief, for each individual test variable, a z-score was calculated from the

mean and standard deviation of the cohort. A domain z-score was then calculated by summing the z-scores for all tests within that domain and renorming the resulting aggregate score. Individual test variables along with their cognitive test assignments are shown in Table S1.

Statistical Analyses

Primary analyses: Linear mixed-effects modeling[27] was performed on the UPenn cohort to evaluate the effect of plasma EGF on cognitive decline over time, adjusting for age and sex. In the ADNI cohort, Cox proportional hazards models were used to estimate the relative risk of conversion from AD-MCI to dementia for different baseline levels of EGF, adjusting for age and sex. In the PARS cohort, a multivariate linear model adjusting for age and sex was used to evaluate the cross-sectional association between EGF and performance in the five aforementioned cognitive domains. In the case of the UPenn PD cohort, covariates were chosen for the primary model to replicate our prior analyses.[11] In the case of the ADNI and PARS cohorts, covariates were chosen to match the PD analysis.

Secondary analyses: In the UPenn PD cohort, we additionally evaluated the effect of adjusting for baseline cognitive performance (MoCA) or motor impairment (UPDRS-III) in order to further understand the basis of the associations observed. In the ADNI cohort, we additionally investigated (1) baseline cross-sectional differences in EGF among NC, AD-MCI, and AD individuals, (2) the effect of omitting or adding further covariates such as baseline MMSE score on model results, and (3) the effect of baseline plasma EGF on change in MMSE over time, using linear mixed-effects models adjusted for age and sex, with or without additional adjustment for baseline MMSE score. In the PARS cohort, we additionally investigated the relationship between EGF and cognitive domain performance (in linear models adjusting for age and sex) within the subgroup at increased risk for development of PD (hyposmic individuals with putaminal dopamine transporter (DAT) binding <80% expected for age), as the PARS cohort is an asymptomatic, rather than prodromal, cohort. We also evaluated the effect of adjusting for DAT binding levels in PARS individuals as an early indicator of whether associations between plasma EGF and cognitive domain performance in PARS individuals are more or less likely to be mediated by dopaminergic systems.

For exact details of each analysis (covariates included, statistical methods used in each situation), please see the Data S1 for R-scripts.

Two-tailed *P*-values are reported for all statistical analyses. Analyses were performed in R, and R-scripts are available in the Data S1.

Results

Low baseline plasma EGF levels predict cognitive decline in PD

Two hundred and thirty-six PD subjects from UPenn were enrolled and followed for up to 5 years (Table 3). Using linear mixed-effect models, we asked if lower plasma EGF levels predicted future cognitive decline. In models adjusted for age and sex, we found a significant association between lower baseline EGF levels and faster rate of cognitive decline ($P = 0.048$). Further adjusting for baseline, MoCA score did not affect our result ($P = 0.014$).

As various risk factors for cognitive decline have been described in PD, including severity and type of motor impairment,[28] we further asked whether EGF correlated with motor symptoms. We found no correlation between plasma EGF levels and scores on the motor portion of the Unified Parkinson's Disease Rating Scale (UPDRS-III) at baseline ($P = 0.682$). Accordingly, adjusting our model

Table 3. Low plasma EGF predicts cognitive decline in PD.

(A) Time	PD Patients with MoCA
Baseline	236
12 months	165
24 months	104
36 months	55
48 months	23
60 months	10

(B) Outcome	Covariates	Coefficient β	*P* -value for EGF
Association with annual change in cognition (MoCA)	Age Sex	0.185	**0.048**
Association with annual change in cognition (MoCA)	Age Sex Baseline MoCA	0.207	**0.014**

(A) Number of PD patients with various lengths of follow-up in our University of Pennsylvania Udall (UPenn) cohort. Montreal Cognitive Assessment (MoCA) performed annually. (B) Linear mixed-effects models demonstrate that baseline levels of plasma EGF predict rates of longitudinal decline in cognition in PD. The coefficient β represents the difference in annual rate of change in the MoCA, for each 1 standard deviation change in baseline EGF. Higher scores on MoCA represent better cognitive function. For example, a subject with a baseline plasma EGF level 1 standard deviation lower than the mean for this sample would be expected to decline ~0.2 points more rapidly per year on the MoCA than a subject with a baseline plasma EGF level at the mean for this sample. Results are shown for models adjusted for age and sex, as well as models adjusted for age, sex, and baseline MoCA score.
Bold indicates significant p-value.

predicting longitudinal cognitive decline from baseline EGF levels to include baseline UPDRS as well as age and sex as covariates did not affect the significant association between baseline EGF levels and rate of cognitive decline ($P = 0.019$).

Low baseline plasma EGF levels predict conversion from amnestic MCI to AD

We next sought to understand whether lower plasma EGF levels predict faster cognitive decline in PD subjects only, or whether these findings could extend to AD as well. To answer this question, we obtained and analyzed data from the ADNI cohort.

In cross-sectional analyses comparing ADNI NC participants ($n = 58$), subjects with amnestic MCI (AD-MCI, $n = 396$), and subjects with AD ($n = 112$), we found that the AD-MCI and AD individuals had significantly lower levels of EGF at baseline ($P = 0.029$, Fig. 1A). Adjusting for age and sex minimally changed the result ($P = 0.056$ for cross-sectional association between EGF and cognitive class), and age and sex did not differ significantly in NC participants versus AD-MCI and AD participants (Table 2) from the ADNI cohort.

Within the AD-MCI individuals of the ADNI cohort, we observed a wide range of plasma EGF levels, suggesting that baseline EGF levels might predict conversion to AD dementia. Of the 396 individuals with baseline EGF measures in the AD-MCI cohort, 387 had available follow-up data, and 203/387 (52%) converted to AD dementia during follow-up. The average time to dementia among individuals who converted to AD was 25.7 months (SD = 18.3 months).

In a Cox proportional hazards survival analysis in these 387 AD-MCI patients, adjusting for age and sex, individuals with the lowest tertile of EGF levels at baseline demonstrated an increased rate of conversion to dementia in follow-up (log-rank test $P < 0.001$, HR: 1.76, 95% CI = 1.33–2.33, Fig. 1B). We next considered the possibility that this apparent effect of EGF might simply be reflecting a difference in cognition between low- versus high-EGF individuals at baseline. However, EGF levels did not correlate with baseline MMSE, and additionally adjusting for baseline MMSE score in our Cox proportional hazards analysis only strengthened the effect (log-rank test $P < 0.001$, HR: 1.87, 95% CI 1.41–2.48 for association between plasma EGF at baseline and risk of conversion from AD-MCI to AD). Omitting adjustment for covariates also did not affect results (log-rank test $P < 0.001$, HR: 1.741, 95% CI = 1.31–2.30).

To further confirm the value of baseline plasma EGF measures in predicting longitudinal change in cognition in these 387 AD-MCI individuals, we employed linear mixed-effects models incorporating MMSE scores from 2444 total visits; each individual was followed at 6-month intervals for up to 96 months. In these longitudinal analyses adjusted for age and sex, we found a significant association between lower baseline EGF levels and faster decline in MMSE ($P = 0.033$). Further adjusting for baseline MMSE score did not alter our results ($P = 0.031$).

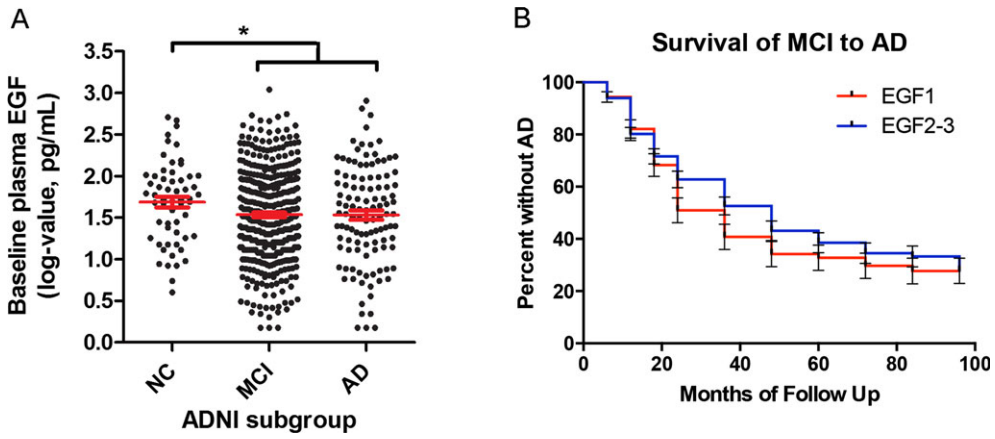


Figure 1. Plasma EGF as a predictive biomarker in amnestic MCI-to-Alzheimer's disease (AD) conversion. At baseline, plasma EGF levels are lower in Alzheimer's Disease Neuroimaging Initiative (ADNI) subjects with amnestic mild cognitive impairment (AD-MCI, $n = 396$), and subjects with (AD, $n = 112$), compared to cognitively normal subjects (CN, $n = 58$). Means +/− SEMs are shown. *$P < 0.05$. Lower baseline levels of EGF correlate with higher risk of conversion to AD among ADNI individuals with AD-MCI ($n = 396$, log-rank test $P < 0.001$, HR: 1.741, 95% CI = 1.31–2.30). Survival curves for months without conversion to AD are shown for AD-MCI individuals with the lowest tertile of baseline plasma EGF levels (red) versus those with higher tertiles of plasma EGF levels (blue). Adjusting for age at plasma sampling and sex in Cox proportional hazards models did not change the result (log-rank $P < 0.001$, HR: 1.76, 95% CI = 1.33–2.33).

Low plasma EGF levels correlate cross-sectionally with visuospatial domain impairment in an asymptomatic cohort at high risk of developing PD

Having shown that lower plasma EGF levels associate with cognitive decline in PD, and that lower EGF levels also characterize AD-MCI individuals at higher risk of cognitive decline and conversion to AD, we next asked whether lower EGF levels correlated with poorer cognition in prodromal PD as well.

To answer this question, we turned to the PARS cohort, in which investigators have intensely characterized a cohort of asymptomatic individuals, some at increased risk for developing PD.[23,29] From an initial pool of more than 5000 individuals, PARS investigators used a sequential biomarker strategy consisting of olfactory testing followed by DAT imaging to characterize lower risk versus higher risk groups. In these at-risk individuals, putaminal DAT uptake determined by [^{123}I] β-CIT SPECT has been used as a measure of DAT density, a proxy for striatal dopaminergic neuron terminal integrity.[30]

We measured plasma EGF levels by ELISA in 165 individuals from the PARS cohort, to assess whether EGF levels associated with performance on any of the five cognitive domains – executive function, language, memory, attention, visuospatial – tested by a comprehensive neuropsychological battery.

When all PARS subjects, regardless of DAT uptake and olfactory testing results, were considered as a group, no significant correlations between EGF and cognitive domain scores were observed (Fig. 2A). However, in the subset of prodromal individuals at highest risk for development of PD (hyposmic individuals with putaminal DAT uptake <80% of age-expected value, $n = 35$), we found that lower plasma EGF levels correlated with poorer performance on visuospatial domain tasks (Fig. 2B), in a linear regression model adjusting for age and sex (nominal $P = 0.045$). Further adjusting our model for DAT binding levels strengthened the association (nominal $P = 0.010$).

In contrast to these visuospatial domain findings, cognitive performance in the other four domains did not correlate with EGF levels in either the full PARS cohort or the high-risk subgroup (Fig. 2C).

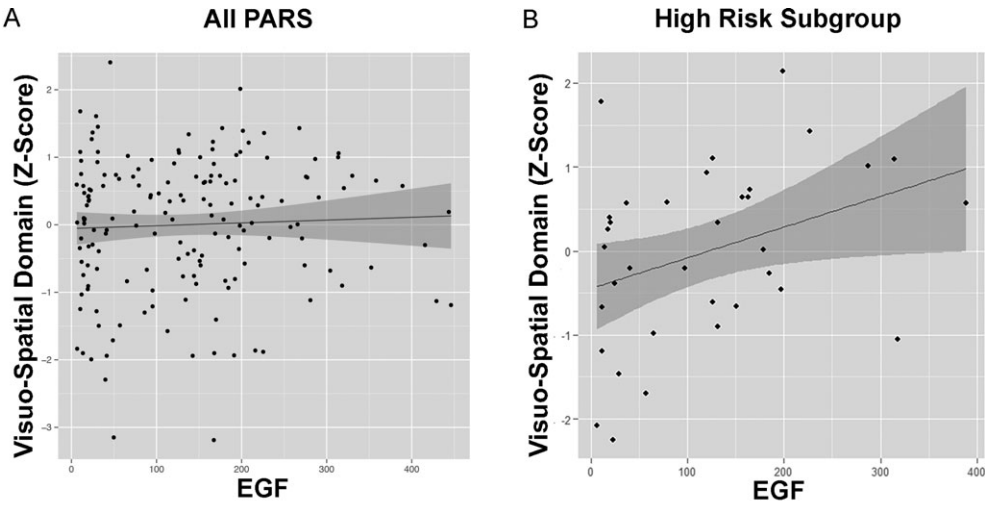
Discussion

In this study, we evaluated the association of plasma EGF levels with cognitive outcome in 236 PD individuals from UPenn, 566 individuals from ADNI, and 165 asymptomatic individuals at risk for PD from PARS. In longitudinal follow-up, we replicated our previous finding that low baseline plasma EGF levels predict cognitive decline in PD. Moreover, in ADNI subjects, plasma EGF levels were decreased in AD-MCI and AD individuals compared to neurologically normal controls, and lower baseline plasma EGF levels also predicted a higher risk of conversion to AD. Finally, in PARS subjects at highest risk for development of PD, low EGF correlated with poorer visuospatial task performance. Taken together, our data suggest that low plasma EGF levels predict accelerated rates of neurodegeneration in both AD and PD, possibly through alterations in shared neurotrophic signaling pathways.

We previously nominated plasma EGF as a biomarker predicting cognitive decline in PD, emerging from an unbiased screen of ~100 proteins.[11] In our initial study, however, the longitudinally followed patient sample was small, consisting of only 49 subjects, and a subsequent replication by an independent group also suffered from a limited sample size of 65.[12] Here, we replicate our finding in 236 new PD patients followed for up to 5 years, a key result because demonstration of the robustness of initial findings is an important, but often unsuccessful, aspect of biomarker research.

Broadening the scope of plasma EGF as a predictive biomarker is our demonstration here that low EGF levels are also predictive of the transition from AD-MCI to AD. Indeed, we found that AD-MCI individuals with the lowest baseline levels of plasma EGF expression had ~80% increase in their risk of conversion to AD, compared to AD-MCI individuals with higher levels of EGF expression. Prior to this study, plasma EGF levels have been reported in small cohorts of AD patients, with conflicting results in different studies with respect to the direction by which AD patients differ from controls.[31,32] In order to reach a more definitive answer, we evaluated 387 AD-MCI subjects from the 55-site international ADNI cohort, in longitudinal analyses with follow-up for an average of 3 years. We note further that our result was robust to changes in modeling paradigms. That is, we continued to find significant associations between lower baseline plasma EGF levels and faster cognitive decline whether we (1) adjusted or did not adjust for various covariates, (2) used Cox proportional hazards analyses to evaluate risk for conversion to AD dementia, or (3) used linear mixed-effects models to evaluate baseline EGF as a predictor of longitudinal rate of change in MMSE. We thus add plasma EGF to a very small number of biochemical biomarkers – CSF tau and beta-amyloid chief among them – that have demonstrated significant predictive value in an international cohort of AD patients.[33,34]

We find that low plasma EGF levels may correlate cross-sectionally with visuospatial impairment in the highest-risk individuals from the asymptomatic PARS



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Domain	Coefficient β (EGF)	Adjusted R^2	P-value (EGF)
Executive Function	0.098	0.085	0.609
Language	0.199	-0.076	0.338
Memory	0.208	0.048	0.301
Attention	0.127	0.262	0.475
Visuo-spatial	0.505	0.222	**0.010**

Figure 2. Plasma EGF correlates with visuospatial domain performance in asymptomatic individuals at high-risk for PD. A, B. In the full PARS cohort, comprising asymptomatic individuals at high- and low-risk for development of PD (n = 165), plasma EGF levels did not correlate with visuospatial domain performance (Panel A). In high-risk asymptomatic individuals (defined by hyposmia and putaminal dopamine transporter uptake <80% expected for age, n = 35) from the Parkinson's Associated Risk Study (PARS) cohort, plasma EGF levels correlated with performance in the visuospatial domain, with higher EGF associating with better performance (Panel B). Each dot represents one subject. EGF levels are represented on the *X*-axis in pg/ml, and visuospatial domain performance is represented on the *Y*-axis as standard deviations from the mean. (C) Association of plasma EGF levels with performance in five cognitive domains was tested in linear models adjusted for age, sex and dopamine transporter (DAT) binding levels, using the high-risk asymptomatic individuals from the PARS cohort. Visuospatial domain performance correlated with plasma EGF levels, with higher EGF associated with better performance. No correlations were observed between plasma EGF levels and performance in the other four domains. The coefficient β represents the difference in performance in each of the cognitive domains, for each 1 standard deviation change in baseline EGF.

cohort. This finding is certainly less definitive than our longitudinal results for cognitive decline in overt PD and for the MCI-to-AD conversion, as it emerges from subgroup analyses and may not survive Bonferroni correction, depending on which covariates are used in the model. However, two aspects of our result merit discussion. First, EGF levels correlate with visuospatial domain performance, but not with performance in other cognitive domains. Second, we find this association between EGF levels and visuospatial domain performance in only the highest-risk individuals within the asymptomatic PARS cohort, and the association is more significant when DAT binding levels are included as a covariate. Intriguingly, multiple studies have demonstrated that visuospatial domain impairment is a frequent feature of the mild cognitive impairment associated with PD (PD-MCI), even in its earliest stages.[35–37] The fact that the relationship between plasma EGF and visuospatial domain impairment is only seen in the highest-risk asymptomatic PARS cohort individuals could be due to chance. However, our results may also represent the biomarker signature of one of the earliest phases of PD-MCI, emerging even prior to a formal diagnosis of PD. Longitudinal findings from the PARS cohort (which is actively being followed) will be important in providing further support or refuting this possibility.

While biomarkers that can be measured in the blood are attractive for many practical reasons, the finding of a correlation between a peripheral protein and a central nervous system (CNS) disease state begs the question of

whether evidence exists for the peripheral protein in question playing a significant role in relevant CNS processes. In the existing literature, EGF signaling pathways have been reported to play a role supporting the survival of dopaminergic neurons in multiple animal models of PD[38,39] as well as in cell culture.[40,41] More generally, EGF pathways have been reported to mediate the proliferation of neural precursor cells in the Subventricular zone (SVZ).[16,17] Intriguingly, both dopaminergic signaling and signaling via the soluble secreted form of the amyloid precursor protein (sAPP) have been reported to influence this EGF-mediated SVZ neuronal proliferation.[16,17,42] Taken together, these data suggest that both PD- and AD-relevant processes might influence EGF signaling pathways; these changes in EGF signaling might, in turn, affect the survival of existing neurons or the process of adult neurogenesis.

Several limitations to this study should be considered in interpreting our results. First, while intriguing, the relationship observed between low EGF plasma levels and visuospatial domain impairment in the PARS cohort is based on a limited number ($n = 35$) of high-risk individuals, and the significance of the association barely survives correction for multiple cognitive domain testing (nominal $P = 0.010$ for one out of five domains tested). As a consequence, this result should be considered preliminary, and replication in a larger set of extensively characterized prodromal PD individuals, cohorts of which are being assembled now, may be needed to fully understand the role of plasma EGF as a prodromal PD biomarker. More generally, the data presented here are correlative; additional mechanistic studies in progressive animal models of AD and PD would be needed to understand the role of EGF-based trophic support as a potential therapeutic avenue in these diseases.

With these caveats in mind, however, we have evaluated EGF plasma levels here in nearly 1000 unique individuals at prodromal and manifest stages of PD and AD, demonstrating that low EGF levels robustly predict poorer cognition in both of these diseases. Moreover, EGF as a candidate biomarker emerged as the top hit from an unbiased screen of ~100 plasma proteins.[11] Our findings support the general approach of unbiased screening for lead discovery,[43] which may in turn lead to both practical biomarker development and indications of potential routes for therapeutic exploration. From an immediate practical perspective, our study suggests that a relatively simple blood test may be able to predict which individuals are at highest risk to cognitively decline in both PD and AD-MCI. From a therapeutic perspective, our study suggests that further investigations into whether enhanced EGF-based trophic support may slow neurodegeneration may be warranted. With over 6 million people suffering from AD and PD in the US alone,[44] and no FDA-approved disease-modifying therapies for either disease, both immediate practical biomarkers and delineation of novel therapeutic approaches are greatly needed.

Author's contributions

EGF plasma measurements were performed by TLU, and statistical analyses were performed by NSL, CRS, HRC, and ACP. Samples were collected by KM, MS, AS, DW, ACP, and JQT. ACP conceived of and provided financial support for the study. The manuscript was written by NSL, HRC, and ACP, with editing for important intellectual content by all authors. Data used in preparation of this article were obtained from the ADNI database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data, but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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Conflicts of Interest

The authors declare no competing financial interests.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Data S1. RBM3 mixed-effects model scripts.
Table S1. Neuropsychological tests in PARS cohort. Supplementary R-scripts (statistical analyses).

