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Plasma EGF levels predict cognitive decline in Parkinson's Disease

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Abstract

Objective—Most people with Parkinson's disease (PD) eventually develop cognitive impairment (CI). However, neither the timing of onset nor the severity of cognitive symptoms can be accurately predicted. We sought plasma-based biomarkers for CI in PD.

Methods—A discovery cohort of 70 PD patients was recruited. Cognitive status was evaluated with the Mattis Dementia Rating Scale-2 (DRS) at baseline and on annual follow-up visits, and baseline plasma levels of 102 proteins were determined with a bead-based immunoassay. Using linear regression, we identified biomarkers of CI in PD, i.e. proteins whose levels correlated with cognitive performance at baseline and/or cognitive decline at follow-up. We then replicated the association between cognitive performance and levels of the top biomarker, using a different technical platform, with a separate cohort of 113 PD patients.

Results—Eleven proteins exhibited plasma levels correlating with baseline cognitive performance in the discovery cohort. The best candidate was epidermal growth factor (EGF, $p < 0.001$); many of the other 10 analytes co-varied with EGF across samples. Low levels of EGF not only correlated with poor cognitive test scores at baseline, but also predicted an eightfold greater risk of cognitive decline to dementia-range DRS scores at follow-up for those with intact baseline cognition. A weaker, but still significant, relationship between plasma EGF levels and cognitive performance was found in an independent replication cohort of 113 PD patients.

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Interpretation—Our data suggest that plasma EGF may be a biomarker for progression to CI in PD.

Keywords

Epidermal growth factor; EGF; Parkinson's Disease; Parkinson's Disease with Dementia; Biomarker; Plasma

INTRODUCTION

Parkinson's disease (PD) is a common neurodegenerative disease affecting dopaminergic neurons of the substantia nigra, resulting in symptoms of bradykinesia, rigidity, and tremor. Over time, however, the disease process spreads throughout many brain regions,¹ and cognitive symptoms almost inevitably develop.^{2,3, 4} Indeed, up to 83% of PD patients develop dementia during their disease course.³⁻⁵

The development of PD with dementia (PDD) or cognitive impairment (CI) is a significant transition for patients and families,⁶ and adds to the cost of care,⁷ yet its occurrence is unpredictable.⁸ While clinical measures^{9, 10} and genotype at the apolipoprotein E gene (*APOE*)¹¹ or microtubule associated protein tau gene (*MAPT*)¹² may be broadly informative of the risk of developing CI in PD, and PDD may demonstrate reduced CSF levels of A β 1-42 (Siderowf *et al*, in press),¹³ a plasma-based biomarker for the risk of CI or dementia in PD, usable at the individual level, is urgently needed.

In the present study, we evaluated levels of 102 plasma-based proteins for correlation to cognitive performance at baseline and over a median follow-up period of 21 months. We identified 11 potential biomarkers of CI in PD, with epidermal growth factor (EGF) demonstrating the most robust signal. Strikingly, low EGF levels were also predictive of a greatly increased risk of conversion to PDD-range cognition during follow-up and may implicate EGF in a new pathway in the development of dementia in PD.

METHODS

Plasma sampling and biomarker quantitation

For the discovery set, 70 patients aged 60 years or older with a diagnosis of idiopathic PD based on British Brain Bank criteria¹⁴ were recruited to the University of Pennsylvania (UPenn) Udall Center without bias towards high or low cognitive function, with the exception that subjects meeting criteria for dementia with Lewy bodies (DLB)¹⁵ were excluded. For the replication set, 113 additional patients were subsequently recruited in the same manner as the discovery set (next consecutive recruits to our study center). Whole blood samples from all patients were obtained under IRB approval (EDTA 10.8mg, 5mL tubes), placed immediately on ice, spun down for plasma aliquotting (3000rpm, 5min, 4°C, 0.5mL aliquots), and frozen at -80°C within the same day. Plasma aliquots were then stored at -80°C until analysis. Collection site and specimen processing were uniform for all samples in both patient cohorts.

Simultaneous screening of 151 proteins by multiplex immunoassay on the Human DiscoveryMAP panel using a Luminex100 platform was then performed for discovery cohort samples, in one batch, by Rules-Based Medicine, Inc. (RBM, Austin, TX), as previously described,¹⁶ and reviewed recently (Hu *et al*, in press). Additional details are available from RBM (<http://www.rulesbasedmedicine.com>) and in supplementary methods. Of the 151 proteins in the RBM Human DiscoveryMAP panel, 102 proteins passed our quality control measures (<20% of samples below lowest reliable value as given by RBM,

<40% coefficient of variation (CV) across technical triplicates) and were used (see Supplementary Table 1).

For confirmation of EGF values, separate aliquots of samples previously run on the multiplex immunoassay were quantified on an EGF enzyme-linked immunosorbent assay (ELISA, R&D Systems, Minneapolis, MN). The same ELISA assay was used to measure plasma EGF levels in the replication cohort.

Cognitive tests

The Mattis Dementia Rating Scale-2 (DRS)¹⁷ was used to evaluate cognition in study patients.^{18,19,20,21} A raw score was obtained and adjusted for age as previously described.²² We used an age-adjusted score cut-off of ≤ 5 for cognitive performance in the PDD range, following recommended criteria in the DRS manual,¹⁷ also validated in PD patients.¹⁹ All subjects had baseline DRS testing within 6 months of plasma draw; 61/70 subjects had DRS testing on the same day as the plasma draw – the 9 individuals who were tested on a different day fell into all 4 quartiles of EGF values. 61/70 subjects had subsequent DRS testing during a median follow-up of 21 months.

APOE genotyping

DNA was extracted from EDTA blood samples using commercial reagents (FlexiGene, Qiagen, Valencia, CA). Two single nucleotide polymorphisms (SNPs) (rs7412 and rs429358) in *APOE* were genotyped using allelic discrimination assays with TaqMan reagents (Applied Biosystems, Foster City, CA) on an ABI 7500. The *APOE* genotypes ($\epsilon 2$, $\epsilon 3$, and $\epsilon 4$) were assigned by incorporating the genotyping results from both SNPs into an algorithm.

Statistical analyses

Linear regression analyses evaluating the association of levels of each protein to age-adjusted DRS scores were performed in R. Full details are in supplementary methods. In brief, the model used for discovery screening designated age and gender as covariates and evaluated the association of each protein individually to DRS scores. For the top 11 proteins, we further evaluated the association between each protein and the DRS score in models incorporating additional covariates such as UPDRS motor score or disease duration since these factors are known to be associated with cognitive impairment. Of note, for EGF, our top analyte, the best multivariate model by a forward stepwise approach designated EGF as the independent variable, age-adjusted DRS as the dependent variable, and age and sex as covariates with no interaction terms. Hence, this was used as the final model for the discovery set. In addition, for EGF, we performed secondary analyses incorporating medications (as yes/no categorical factors) and *APOE* genotype as covariates to evaluate whether these factors affected the association between EGF levels and cognitive performance.

The Partek Genomics Suite was used to perform hierarchical cluster analysis (Euclidean distance) of co-expression among the top 11 proteins, and to generate graphics (Partek GS, copyright 2010, St. Louis, MO). Survival curves were compared with log-rank tests. To evaluate the effects of baseline DRS performance, age, and gender on the relationship between EGF quartile and conversion to PDD-range DRS, Cox proportional hazards models were used. All statistical tests were two-sided.

In the replication cohort, as in the discovery cohort, linear regressions were used to evaluate the relationship between DRS performance and EGF levels, as well as other potential variables (age, sex, UPDRS motor score) affecting cognitive performance. A forward

stepwise approach was again used to determine the final multivariate model with EGF specified as the independent variable, age-adjusted DRS as the dependent variable, and age, sex, UPDRS motor score, and their interaction terms as possible covariates. The final model, with an R^2 value of 0.28, incorporated sex, UPDRS motor score, EGF, and their interaction terms. An alternative model substituting Hoehn and Yahr stage for UPDRS motor score performed similarly.

RESULTS

Study cohorts

70 PD patients were used in the discovery phase of the study, and 113 patients were used in the replication phase, with the total 183 patients representing consecutive study recruits. In the initial cohort of 70, 16 (23%) had cognitive scores within the PDD-range (age-adjusted $DRS \leq 5$), and 54 did not (age-adjusted $DRS > 5$); age, gender, age at disease onset, disease duration, UPDRS motor scores, use of dopaminergic agents, and *APOE* genotypes were similar between these two groups (Table 1).

In the replication cohort, 13/113 (12%) had PDD-range cognitive scores. Unlike in the discovery cohort, in this case, the PDD-range individuals were more likely to be male ($p=0.016$), significantly older ($p=0.029$), with a longer disease duration ($p=0.002$) and higher UPDRS motor scores ($p=0.023$) than the non-demented individuals. They were also less likely to be on dopamine agonists ($p=0.002$); use of levodopa and *APOE* genotypes were similar to non-demented individuals (Table 1).

Identification of biomarkers for cognitive impairment in PD

Using our discovery cohort, we evaluated levels of 102 proteins individually for correlation with age-adjusted DRS, using linear regression in a model adjusting for age and gender. Eleven proteins showed correlations between plasma levels and DRS score ($p < 0.05$), with EGF ($p < 0.001$) and CD40 ligand ($p = 0.006$) demonstrating the most significant associations (Table 2). Moreover, most of these 11 proteins co-varied across samples (Figure 1), with EGF, CD40 ligand, thrombospondin-1, PAI-1, and PDGF forming one cluster with particularly correlated expression (Figure 1). In addition, the association between cognitive test performance and plasma protein levels persisted for EGF, CD40 ligand, and heparin-binding EGF (HBEGF) in models that accounted for either disease duration or UPDRS-motor scores, in addition to age and gender (Table 2). Interestingly, HBEGF expression was not correlated with EGF expression, despite both demonstrating relationships with cognition, implying differential regulation of these two proteins. Finally, plasma levels of the soluble receptors for EGF and CD40 ligand – EGF receptor (EGF-R) and CD40 – were neither associated with DRS scores, nor correlated with expression of their ligands (Table 2, Figure 1).

Plasma EGF as a candidate biomarker for cognitive impairment in PD

Following this initial screen, we focused on EGF as the top analyte correlated with cognitive performance in PD. Accordingly, we further investigated aspects of EGF as a candidate biomarker for CI in PD. To do this, we used an enzyme linked immunosorbent assay (ELISA) to measure plasma EGF levels on 24 samples from our discovery cohort with same-day duplicate plasma aliquots. Cross-platform correlation of EGF levels by ELISA was excellent across the 24 samples (r^2 0.76, Figure 2a), indicating that EGF is technically robust as a biomarker for CI in PD.

In the initial analyses, DRS scores were treated as a continuous variable. Dichotomizing scores into PDD-range (age-adjusted $DRS \leq 5$) and non-PDD-range (age-adjusted $DRS > 5$),

we evaluated whether levels of our top plasma analyte (EGF) could serve as a classifier for PDD-range DRS. However, trade-offs between specificity and sensitivity limited the utility of EGF as a classifying biomarker for baseline PDD-range performance, with a maximum classification accuracy of 79%.

Plasma EGF levels predict cognitive decline in PD

Follow-up DRS testing was available for 61/70 discovery samples, with a median follow-up of 21 months and interquartile range (IQR) of 13-26 months. We next determined if baseline EGF levels predicted conversion from non-PDD-range DRS scores to PDD-range DRS scores (i.e. ≤ 5) among subjects with age-adjusted DRS scores > 5 at baseline. Strikingly, survival analyses of time to PDD-range showed markedly different outcomes for those subjects with the lowest plasma EGF levels (Figure 2b). Specifically, non-PDD-range subjects with EGF levels in the lowest quartile (Quartile 1, Figure 2c) were eight times more likely to convert to PDD-range, with a median conversion time of 14 months ($p < 0.001$, hazard ratio 8.34, 95% CI 4.26-122.90). The other three quartiles of EGF levels did not differ significantly from each other (Figure 2b). The association between EGF quartile and risk for conversion persisted in models adjusting for age and baseline DRS score ($p = 0.033$ for EGF quartile, hazard ratio 4.95, 95% CI 1.14-21.74, Supplementary Figure 1); or age, gender, and baseline DRS score ($p = 0.037$ for EGF quartile, hazard ratio 4.88, 95% CI 1.10-21.74).

APOE genotype and medications do not affect the association between EGF levels and cognitive performance

We next analyzed the effects of specific medications (levodopa, dopamine agonists, antipsychotic medications, anti-depressant medications, statins, cholinesterase inhibitors, memantine, and benzodiazepines) and *APOE* genotype on our findings. We found no effect of these variables on the association between EGF levels and DRS performance (Table 3).

Correlation of plasma EGF levels and cognitive performance persists in a replication cohort

To add confidence to our finding of a correlation between EGF levels and cognitive performance in the discovery cohort, we recruited an additional 113 PD patients, measured plasma EGF levels by ELISA, and tested cognitive performance with the DRS. While there were significant, unforeseen, differences in the clinical characteristics of this second cohort, the correlation between plasma EGF levels and age-adjusted DRS scores persisted ($p = 0.035$), with the same directionality. In the replication cohort, both gender and degree of motoric impairment (as reflected in UPDRS motor score) were significantly associated with cognitive performance, and the best multivariate model included these two factors, as well as interaction terms, with EGF levels as predictors of age-adjusted DRS score (Figure 3).

DISCUSSION

In the present study, we evaluated cognitive performance and plasma levels of 102 proteins in an initial discovery cohort of 70 PD patients. We identified 11 potential biomarkers of CI in PD, with EGF as our top biomarker, showing promise as a predictor of cognitive decline in PD. We then replicated the association between plasma EGF levels and cognitive performance in a separate cohort of 113 PD patients, using a different technical platform for measuring EGF.

Plasma EGF demonstrates several characteristics that make it attractive as a biomarker for CI in PD. First, as shown in our cross-platform comparison and use of two different technical assays in the discovery and replication cohorts, measurement of EGF levels is

robust to differing assay techniques. Second, despite demographic and clinical differences between our discovery and replication cohorts, the relationship between plasma EGF levels and cognitive performance persisted. This last point may be particularly important, as many potential biomarkers fail to replicate in follow-up studies, presumably due to the considerable “noisiness” of clinical data, which leads to both false positives and false negatives.

In addition, the fact that plasma EGF levels have potential for serving as a predictor of cognitive decline in PD has both practical and mechanistic implications. On a practical level, such a predictive biomarker might prove quite useful in identifying at-risk populations for clinical intervention with trial therapeutic agents. On a mechanistic level, the fact that EGF levels are low in PD with CI and in PD at highest risk of developing CI suggests that EGF pathways may be implicated mechanistically in the development of CI and dementia in PD. Indeed, EGF has been reported as a neurotrophic factor supporting both adult subventricular zone neurons²³ and midbrain dopaminergic neurons²⁴ in models of PD and in human PD patients. Moreover, EGF-R signaling pathways may regulate the survival of midbrain dopaminergic neurons in PD models.²⁵ In our study, levels of both EGF and HBEGF – another protein reported to support dopaminergic neurons²⁶ – were correlated with cognitive function; yet expression levels of these two proteins were not correlated with each other across samples. This finding suggests that the effect observed (1) may be mediated by EGF-R, for which both EGF and HBEGF can serve as ligands, and (2) is not due to a confounder, which might be expected to affect EGF levels and HBEGF levels similarly across cases. It should be noted that while EGF-R levels measured in this study were not correlated with cognitive performance, our method would only quantify circulating EGF-R, which may not reflect levels of cell-bound receptor.

Given the association between plasma EGF levels and DRS scores, why was EGF not a better classifier for baseline cognition? Close scrutiny of patterns of EGF expression in our cohort reveals that many samples mis-classified (using low EGF levels) as falling into the PDD-range in fact “convert” to PDD-range cognitive performance in the follow-up period (Figure 1, green arrows). Thus, the very fact that low EGF levels are predictive of future cognitive decline may hamper utility as a classifying biomarker for baseline cognition. The implication of this observation is that low EGF levels precede the development of dementia in PD and that EGF signaling may be involved mechanistically in progression of cognitively normal PD patients to PDD.

Our replication cohort differed in several important ways from our discovery cohort. Both cohorts were recruited in the same manner, to the same center, as consecutive study subjects; their differences may reflect characteristics of patients more likely to join a research study earlier vs. later. In any case, the relationship between plasma EGF and cognition was observed in both cohorts. It is worth noting, however, that the correlation only achieved statistical significance in the latter cohort after accounting for effects from gender and motoric impairment. While it is certainly possible that this is due to Type I error in our discovery cohort, the more likely interpretation is that thorough analysis of effects of other factors on cognition in PD will be needed in follow-up studies, given the considerable variation among human subjects.

Several caveats should be considered in interpreting our results. First, while we were able to replicate the cross-sectional association between EGF levels and cognitive performance, follow-up in the replication cohort (recruited after the discovery cohort) has been too short to assess whether the finding of low EGF levels as predictive of cognitive decline is also seen. Thus, future studies evaluating more PD patients with long follow-up periods would be a valuable addition to the data presented here.

One question that arises from our current study is whether the correlation between low EGF levels and poor cognitive performance is specific to PD. While a thorough consideration of this question is outside the scope of this study, we found no association between EGF levels and *APOE* genotype, a risk factor for Alzheimer's Disease (AD), and we have not found EGF levels to differ significantly between AD patients and normal controls in CSF,²⁷ or in plasma (manuscript in prep).

In summary, we used an unbiased screen of 102 proteins to identify potential biomarkers for CI in PD. Eleven proteins emerged from our screen, many showing co-expression with the top analyte, EGF. The correlation between plasma EGF levels and cognitive performance replicated in an additional cohort of 113 patients, on a different technical platform. Finally, low levels of EGF were predictive of an eightfold greater risk of conversion from non-PDD-range cognitive performance to PDD-range cognitive performance, with a median time-to-conversion of 14 months.

While some studies have reported CSF biomarkers as potential indicators of risk for development of PDD,¹³ to our knowledge, the current result is the first plasma-based, non-genetic risk factor for CI in PD. If confirmed by further studies, the measurement of EGF levels may be useful both as a clinical diagnostic tool and in the design of trials aimed at preserving cognition in PD. With PD affecting more than 500,000 individuals in the US alone, and prevalence projected to double in the next 25 years,⁵ the need for such tools cannot be overstated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Braak H, Del Tredici K, Rub U, et al. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging*. 2003; 24:197–211. [PubMed: 12498954]
2. Williams-Gray CH, Foltynie T, Brayne CE, et al. Evolution of cognitive dysfunction in an incident Parkinson's disease cohort. *Brain*. 2007; 130:1787–1798. [PubMed: 17535834]
3. Hely MA, Reid WG, Adena MA, et al. The Sydney multicenter study of Parkinson's disease: the inevitability of dementia at 20 years. *Mov Disord*. 2008; 23:837–844. [PubMed: 18307261]
4. Buter TC, van den Hout A, Matthews FE, et al. Dementia and survival in Parkinson disease: a 12-year population study. *Neurology*. 2008; 70:1017–1022. [PubMed: 18362281]
5. Mayeux R, Denaro J, Hemenegildo N, et al. A population-based investigation of Parkinson's disease with and without dementia. Relationship to age and gender. *Arch Neurol*. 1992; 49:492–497. [PubMed: 1580811]
6. Aarsland D, Larsen JP, Karlsen K, et al. Mental symptoms in Parkinson's disease are important contributors to caregiver distress. *Int J Geriatr Psychiatry*. 1999; 14:866–874. [PubMed: 10521886]
7. Pressley JC, Louis ED, Tang MX, et al. The impact of comorbid disease and injuries on resource use and expenditures in parkinsonism. *Neurology*. 2003; 60:87–93. [PubMed: 12525724]

8. Leverenz JB, Quinn JF, Zabetian C, et al. Cognitive impairment and dementia in patients with Parkinson disease. *Curr Top Med Chem*. 2009; 9:903–912. [PubMed: 19754405]
9. Uc EY, Rizzo M, Anderson SW, et al. Visual dysfunction in Parkinson disease without dementia. *Neurology*. 2005; 65:1907–1913. [PubMed: 16282276]
10. Levy G, Jacobs DM, Tang MX, et al. Memory and executive function impairment predict dementia in Parkinson's disease. *Mov Disord*. 2002; 17:1221–1226. [PubMed: 12465060]
11. Huang X, Chen P, Kaufer DI, et al. Apolipoprotein E and dementia in Parkinson disease: a meta-analysis. *Arch Neurol*. 2006; 63:189–193. [PubMed: 16476806]
12. Goris A, Williams-Gray CH, Clark GR, et al. Tau and alpha-synuclein in susceptibility to, and dementia in, Parkinson's disease. *Ann Neurol*. 2007; 62:145–153. [PubMed: 17683088]
13. Mollenhauer B, Trenkwalder C, von Ahsen N, et al. Beta-amyloid 1-42 and tau-protein in cerebrospinal fluid of patients with Parkinson's disease dementia. *Dement Geriatr Cogn Disord*. 2006; 22:200–208. [PubMed: 16899997]
14. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry*. 1992; 55:181–184. [PubMed: 1564476]
15. McKeith IG, Dickson DW, Lowe J, et al. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. *Neurology*. 2005; 65:1863–1872. [PubMed: 16237129]
16. Spina S, Murrell JR, Huey ED, et al. Corticobasal Syndrome Associated With the A9D Progranulin Mutation. *J Neuropathol Exp Neurol*. 2007; 66:892–900. [PubMed: 17917583]
17. Jurica PJ, L S, Mattis S. Dementia Rating Scale-2 (DRS-2): Professional Manual: Psychological Assessment Resources. 2001
18. Llebaria G, Pagonabarraga J, Kulisevsky J, et al. Cut-off score of the Mattis Dementia Rating Scale for screening dementia in Parkinson's disease. *Mov Disord*. 2008; 23:1546–1550. [PubMed: 18546326]
19. Aarsland D, Litvan I, Salmon D, et al. Performance on the dementia rating scale in Parkinson's disease with dementia and dementia with Lewy bodies: comparison with progressive supranuclear palsy and Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. 2003; 74:1215–1220. [PubMed: 12933921]
20. Reyes MA, Lloret SP, Gerscovich ER, et al. Addenbrooke's Cognitive Examination validation in Parkinson's disease. *Eur J Neurol*. 2009; 16:142–147. [PubMed: 19049504]
21. Dubois B, Burn D, Goetz C, et al. Diagnostic procedures for Parkinson's disease dementia: recommendations from the movement disorder society task force. *Mov Disord*. 2007; 22:2314–2324. [PubMed: 18098298]
22. Lucas JA, Ivnik RJ, Smith GE, et al. Normative data for the Mattis Dementia Rating Scale. *J Clin Exp Neuropsychol*. 1998; 20:536–547. [PubMed: 9892057]
23. O'Keefe GC, Tyers P, Aarsland D, et al. Dopamine-induced proliferation of adult neural precursor cells in the mammalian subventricular zone is mediated through EGF. *Proc Natl Acad Sci U S A*. 2009; 106:8754–8759. [PubMed: 19433789]
24. Iwakura Y, Piao YS, Mizuno M, et al. Influences of dopaminergic lesion on epidermal growth factor-ErbB signals in Parkinson's disease and its model: neurotrophic implication in nigrostriatal neurons. *J Neurochem*. 2005; 93:974–983. [PubMed: 15857400]
25. Inoue H, Lin L, Lee X, et al. Inhibition of the leucine-rich repeat protein LINGO-1 enhances survival, structure, and function of dopaminergic neurons in Parkinson's disease models. *Proc Natl Acad Sci U S A*. 2007; 104:14430–14435. [PubMed: 17726113]
26. Farkas LM, Krieglstein K. Heparin-binding epidermal growth factor-like growth factor (HB-EGF) regulates survival of midbrain dopaminergic neurons. *J Neural Transm*. 2002; 109:267–277. [PubMed: 11956950]
27. Hu WT, Chen-Plotkin A, Arnold SE, et al. Novel CSF biomarkers for Alzheimer's disease and mild cognitive impairment. *Acta Neuropathol*.

Figure 1. Expression of proteins with plasma levels associated with cognitive performance

Heat map represents plasma expression of 11 proteins whose levels correlated with baseline cognition (age-adjusted DRS scores), as well as 2 additional proteins (CD40 and EGF-R, purple text) with receptor activity for CD40 ligand and EGF, the top 2 proteins in our screen (red text). Of note, CD40 and EGF-R levels were not correlated with cognition, and plasma levels may not reflect the cell-bound fraction of these receptors. Hierarchical clustering (dendrogram on right side) indicates that many of the 11 cognition-associated proteins co-vary, with CD40 ligand, EGF, PAI-1, Thrombospondin-1, and PDGF representing one cluster with particularly correlated expression. However, HBEGF and EGF do not co-vary across samples, although they may act on the same receptor. Rows in the heat map represent expression levels of each protein (labeled on left side), with red denoting higher expression, blue denoting lower expression, and grey denoting intermediate expression. Columns in the heat map represent individual patients, with columns marked with green bars (top) indicating individuals with baseline age-adjusted DRS ≤ 5 (PDD-range), and those marked with gold bars indicating individuals with baseline age-adjusted DRS > 5 (non-PDD-range). Individuals with baseline PDD-range cognition almost uniformly had low expression for EGF and co-expressed proteins (yellow box). Green arrows (top) indicate individuals with baseline non-PDD-range cognition who converted to PDD-range cognition during follow-up.

Figure 2. EGF as a biomarker for cognitive impairment in PD

(a) EGF plasma levels were robust across technical platforms. Duplicate aliquots of 24 plasma samples were quantified for EGF levels by multiplex immunoassay and by traditional ELISA. Readings by multiplex immunoassay (EGF-RBM, X-axis) were plotted against readings by ELISA (EGF-ELISA, Y-axis). Cross-platform correspondence was excellent, as indicated by an r^2 value of 0.76. Values are shown in pg/mL. (b) Low plasma EGF levels were predictive of conversion from non-PDD-range cognitive performance (age-adjusted DRS>5) to PDD-range cognitive performance (age-adjusted DRS≤5). Follow-up DRS scores were available for 49/54 patients with non-PDD-range baseline cognition. Survival curves of time to PDD-range cognitive performance are shown for those with the lowest quartile of EGF expression (quartile 1, red line), as well as those in EGF quartiles 2 (blue line), 3 (green line), and 4 (purple line). Outcomes for EGF quartile 1 differed significantly from the other 3 quartiles, demonstrating an eightfold higher risk of conversion to PDD-range cognition ($p<0.001$, hazard ratio 8.34, 95% CI 4.26-122.90) with a median time-to-conversion of 14 months. (c) Numbers of individuals (N), EGF values (median, full range in pg/mL) for each EGF quartile are shown among those patients with baseline age-adjusted DRS>5. In addition, the number of individuals converting to an age-adjusted DRS≤5 within the follow-up period is shown for each EGF quartile; follow-up periods (median months, IQR = interquartile range) were similar among the quartiles.

Figure 3. Correlation of plasma EGF and age-adjusted DRS score in an independent replication cohort

(a) Graphical representation of multivariate model used in replication cohort. In the second cohort of 113 PD patients, gender and UPDRS motor scores differed significantly between those individuals with PDD-range cognitive performance (more likely to be male and have high UPDRS motor scores), and those with non-PDD range cognitive performance. Taking into account these effects, low plasma EGF levels were predictive of lower DRS scores in women across all ranges of UPDRS motor scores, and men with low UPDRS motor scores. Red denotes females, and blue denotes males. Solid lines denote individuals with low UPDRS motor scores, and dashed lines denote individuals with high UPDRS motor scores.

(b) A forward stepwise approach was used to determine the final multivariate linear regression model, which specified plasma EGF values as the independent variable, with gender, UPDRS motor scores (UPDRS), and interaction terms as covariates.

Clinical features of Parkinson's patients with (DRS≤5) and without (DRS>5) significant cognitive impairment

Table 1

Discovery cohort: 70 patients had plasma samples drawn within 6 months of testing with the Mattis DRS-2. Of these patients, 16 (23%) had age-adjusted DRS scores of 5 or less, indicating PDD-range performance. Age at plasma draw, age at disease onset, disease duration, UPDRS motor score, gender, medication regimens, and *APOE* genotypes were not significantly different between the two groups.

Replication cohort: A subsequent set of 113 consecutive patients had plasma samples drawn within 6 months of testing with the Mattis Dementia Rating Scale (DRS). Of these patients, 13 (12%) had age-adjusted DRS scores of 5 or less, indicating PDD-range performance. Age at plasma draw, disease duration, UPDRS motor score, gender, and use of dopamine agonists differed between the two groups. Age at onset, use of levodopa, and *APOE* genotypes were not significantly different between the two groups. Of note, medication information was available for 12/13 patients with DRS≤5, and 91/100 patients with DRS>5. *APOE* genotypes were available for 12/13 patients with DRS≤5, and 88/100 patients with DRS>5.

	Discovery cohort (n=70)			Replication cohort (n=113)		
	DRS≤5	DRS>5	P-value	DRS≤5	DRS>5	P-value
Number (%)	16 (23%)	54 (77%)		13 (12%)	100 (88%)	
Age at Plasma Median yrs (IQR)	74.0 (66.5-78.0)	71.0 (67.0-76.0)	0.204	76.0 (72.0-78.0)	69.5 (64.0-75.0)	0.029
Age at Onset Median yrs (IQR)	60.0 (57.0-71.0)	63.0 (57.0-68.0)	0.899	64.0 (56.5-67.8)	62.0 (58.0-68.0)	0.875
Disease Duration Median yrs (IQR)	7.0 (4.5-13.5)	7.0 (4.5-11.0)	0.806	11.0 (9.0-16.0)	6.0 (3.0-9.0)	0.002
UPDRS motor Median (IQR)	18.5 (6.5-26.3)	21.0 (12.4-24.0)	0.768	31.0 (18.0-38.0)	19.5 (11.8-26.0)	0.023
Male:Female	13:3	44:10	0.983	12:1	60:40	0.030
% on L-dopa	56%	57%	0.934	100%	88%	0.923
% on Dopa-agonist	38%	52%	0.313	17%	64%	0.003
<i>APOE</i> E2/E3	1	8		0	8	
E3/E3	10	36	0.503	8	63	0.480
E3/E4	5	9		4	16	
E4/E4	0	1		0	1	

IQR = interquartile range.

Table 2
11 plasma proteins with levels significantly associated with baseline cognitive performance

11 out of 102 proteins screened showed correlations between plasma level and age-adjusted DRS scores (nominal $p < 0.05$) in linear regression models with age and gender as covariates (Model 1). Square of correlation coefficient (R^2) and direction of association (Dir, with positive (+) indicating a higher expression value correlating with higher DRS performance) shown for Model 1. Of these 11 proteins, levels of 3 proteins (CD40 ligand, EGF, and HBEGF, shaded rows) showed significant correlations with age-adjusted DRS scores in two additional models including either disease duration (Model 2) or UPDRS motor scores (Model 3) as additional covariates. EGF = epidermal growth factor. ENA78 = epithelial neutrophil-activating protein 78. FAS = Fas antigen. GROalpha = growth regulated oncogene-alpha. HBEGF = heparin-binding epidermal growth factor. PAI1 = plasminogen activator inhibitor-1. PDGF = platelet-derived growth factor. Note the preponderance of growth factors among proteins associated with cognitive performance.

Plasma protein	R^2	Dir	Model 1 P-value	Model 2 P-value	Model 3 P-value
CD40 ligand	0.192	+	0.006 **	0.023 *	0.033 *
EGF	0.231	+	<0.001 ***	0.008 **	0.012 *
ENA78	0.171	+	0.015 *	0.057	0.055
FAS	0.174	-	0.013 *	0.022 *	0.488
GROalpha	0.172	+	0.014 *	0.114	0.038 *
HBEGF	0.159	+	0.025 *	0.029 *	0.011 *
PAI1	0.174	+	0.013 *	0.052	0.040 *
PDGF	0.165	+	0.019 *	0.044 *	0.084
RANTES	0.166	+	0.019	0.074	0.031
Stem cell factor	0.156	+	0.029 *	0.125	0.063
Thrombospondin1	0.177	+	0.011 *	0.023 *	0.094

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$.

Table 3
Relationship between cognitive performance and EGF plasma values is not affected by medications or ApoE genotype

To evaluate whether the association between EGF levels and DRS scores was affected by factors such as medications or ApoE genotype, each factor was entered as an additional categorical factor in a linear model with age, gender, and EGF levels as continuous variables predicting DRS performance. Adjusting for effects of these additional factors did not affect the relationship between cognitive performance and EGF levels. Of factors analyzed, only the use of anti-depressants was significantly associated with DRS performance, with poorer DRS performance among patients taking anti-depressants. In addition, EGF levels did not differ significantly between patients with/without each medication, as indicated by the non-significant p-values in the last column. EGF levels were compared in groups with/without medication by two-tailed T-tests. EGF levels were compared between different ApoE genotypes (number of ApoE4 alleles) by chi-square testing. AChE inhibitor = cholinesterase inhibitor.

Factor	Linear Regression P-values DRS ~ Age + Gender + EGF + Factor			EGF values +/- Factor (P-values)
	Overall	EGF	Factor	
Statin	0.0006	0.0007	0.1286	0.7827
AChE inhibitor	0.0012	0.0019	0.3924	0.1320
Memantine	0.0011	0.0012	0.3216	0.6038
Anti-psychotic	0.0003	0.0013	0.0588	0.4878
Levodopa	0.0015	0.0011	0.5821	0.8355
Dopamine agonist	0.0016	0.0013	0.8418	0.2635
Anti-depressant	0.0002	0.0027	0.0369	0.1530
Benzodiazepine	0.0004	0.0017	0.0822	0.3439
ApoE4 alleles	0.0016	0.0010	0.9513	0.5822