J Neuropathol Exp Neurol
Vol. 78, No. 8, August 2019, pp. 709–716
doi: 10.1093/jnen/nlz054

ORIGINAL ARTICLE

Plasma Neurofilament Light Chain Levels Are Associated With Cortical Hypometabolism in Alzheimer Disease Signature Regions

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Abstract

Neurofilament light chain (NFL) has been recently introduced as a biomarker of early dementia. 18-Fluorodeoxyglucose positron emission tomography (18F-FDG-PET) is a proxy for regional hypometabolism in Alzheimer disease (AD). Globally normalized 18F-FDG-PET values and levels of NFL and tau were obtained from 149 patients with mild cognitive impairment (MCI) from the baseline cohort of the Alzheimer's Disease Neuroimaging Initiative database. We adopted a stepwise partial correlation model using plasma NFL, plasma tau, CSF NFL, and regional cerebral metabolic rate of glucose (CMRGlc) as main variables, and age, sex, and Alzheimer's Disease Rating Scale (ADAS) as covariates. Significant regions were entered into a stepwise multiple regression analysis to investigate the independent correlation of each biomarker to baseline regional CMRGlc and its progression in patients with MCI. Higher baseline CSF NFL levels correlated with hypometabolism in bilateral precuneal and posterior cingulate cortex. After correction for age, sex, and ADAS score, plasma NFL levels correlated with hypometabolism in bilateral parahippocampal and middle temporal gyri. Cortical hypometabolism in bilateral parahippocampal gyri and right fusiform and middle temporal gyri was independently predicted by higher baseline plasma NFL levels in a multiple regression model. Plasma NFL promises to be an early biomarker of cortical hypometabolism in MCI and for MCI progression to AD.

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The authors declare no duality or conflicts of interest.

*Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (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

Key Words: Alzheimer disease, Neurofilament light chain, Positron emission tomography, Tau.

INTRODUCTION

Investigation of plasma and CSF biomarkers of Alzheimer disease (AD) has reached a turning point. Disease-modifying therapies targeting neurodegeneration in AD have drawn attention toward the significance of early detection of AD (1). We now know that CSF levels of the soluble amyloid beta 42 (Aβ42) start to decline decades before the onset of clinical dementia and that elevated levels of phosphorylated tau (p-tau) in the CSF can differentiate AD from other types of dementia with almost 97% specificity (2). The CSF tau/Aβ42 ratio has been shown to have acceptable specificity and sensitivity in identifying patients with mild cognitive impairment (MCI) with a high risk of conversion to AD (3). Meanwhile, technical difficulties in quantification of CSF Aβ42 levels have subjected CSF Aβ42 to measurement errors (4). Lower plasma levels of Aβ42 were also associated with disease progression in MCI patients (5). However, they have shown poor relevance to cognitive scores and there is no age-specific discriminative limit for Aβ42 in plasma in older individuals (6).

The neurofilament light chain (NFL) is an axonal cytoskeleton protein and a nonspecific biomarker of neurodegeneration that is elevated in the CSF of patients with AD dementia (3). Plasma levels of NFL are shown to have robust predictive values of the cognitive status, both in patients with MCI and AD dementia (7, 8). It has been shown that a combination of CSF Aβ42, p-tau, and NFL levels provides the highest accuracy in differentiating between AD dementia and healthy controls (9).

Position emission tomography (PET) is a gold standard method to detect regional decline in cerebral metabolic rate of glucose (CMRGlc) (10). A growing interest is given to studies investigating convenient and reliable alternatives for neuroimaging makers of dementia, including CSF and plasma markers. NFL has been proposed as a novel and promising biomarker

for early AD (7). Plasma NFL levels have shown relevance to longitudinal changes in regional metabolism in signature hypometabolism regions in AD and they are more convenient to measure than either Aβ42 or tau (7).

Herein, we investigated the correlation between serum and CSF NFL levels with globally standardized CMRGlc identified through 18F-fluorodeoxyglucose (18F-FDG)-PET scan of patients with MCI from the baseline cohort of the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu). We hypothesized that levels of CSF or plasma NFL might correlate with cerebral hypometabolism in signature AD regions and serve as early markers of neurodegeneration. We investigated the independent predictive value of CSF or plasma NFL levels in regional CMRGlc in a multiple regression model with age, sex, cognitive scores, and plasma tau levels as covariates.

MATERIALS AND METHODS

Data Acquisition

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 2003 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). ADNI participants are recruited from across the United States and Canada. We extracted data from screening visits of patients from the baseline ADNI-1 cohort for whom all demographic data, CSF NFL, plasma NFL, and plasma tau levels from screening visit were available. We also extracted the baseline (screening visit) and the 6- and 12-month follow-up 18F-fluorodeoxyglucose (18F-FDG) PET scan data of all patients in the cohort. Our study cohort therefore consisted of 149 patients with MCI, with their baseline CSF NFL, plasma NFL, and plasma tau levels and their 18F-FDG-PET data all acquired at the Banner Alzheimer's Institute (Phoenix, Arizona) and downloaded on December 4, 2018 (http://adni.loni.usc.edu).

In brief, ADNI participants aged between 55 and 90 years and were all willing to undergo all test procedures including 2 lumbar punctures, one at the screening visit and one to complete a 2-year follow-up imaging study, including an 18-FDG-PET scan every 6 months. Patients were excluded if they had high ischemic score in their diffusion-weighted magnetic resonance imaging, a recent change in medications in the 4 weeks prior to the study, less than 6 grades of education or depression. None of the subjects were taking cholinesterase inhibitors, antidepressant medications with anticholinergic properties, neuroleptic agents, antiparkinsonian drugs, chronic anxiolytics, sedative hypnotics, diuretics, or were regular narcotic users at the time or within 4 weeks prior to the screening visit. AD and MCI patients were allowed to take cholinesterase inhibitors and memantine if the dosing had not changed within 4 weeks prior to screening visit. The same drug exclusion criterion applied for 6- and 12-month follow-ups.

Patients with baseline MCI and those who progressed to AD all had complaints of memory impairment. Classification of patients as MCI or AD was based on either of the following criteria: (i) mini-mental state examination (MMSE) score between 24 and 30 for MCI and 20 and 24 for AD, (ii) clinical dementia rating (CDR) score of 0.5 with memory box score $\geq$0.5 for MCI and 0.5 or 1 for AD, or (iii) logical memory II subscale of the Wechsler memory scale below the age-categorized cutoff values. All patients also fulfilled the National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria (11). Full description on subject selection and demographic information of the cohort can be found elsewhere (12).

Plasma Protein Measurements

ADNI samples were analyzed at the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden (13). Human Total Tau Kit (Quanterix, Lexington, MA) on single molecule array (Simoa) HD-1 analyzer (CE marker) was used to measure total plasma tau levels (13). Plasma NFL was similarly assessed using the Simoa technique, which uses monoclonal antibodies in combination with purified bovine NFL used for calibration. All tau and NFL samples were measured in duplicate to increase validity. Analytical sensitivity was $<$1.0 pg/mL for both tau and NFL kits. Plasma tau and NFL values were presented as pg/mL and no plasma sample had NFL or tau levels below the limit of detection.

CSF NFL Measurement

Baseline CSF samples were obtained from all subjects in the morning after overnight fasting, as described in the ADNI procedures manual (http://www.adni-info.org/). CSF samples from each site were stored in polypropylene transfer tubes and shipped to ADNI Biomarker Core laboratory at the University of Pennsylvania Medical Center on dry ice within 1 hour after collection. Aliquots (0.5 mL) were prepared from these samples after thawing, which were then stored at −80°C (14). The sandwich enzyme linked immunosorbent assay (ELISA) method (NF-light ELISA kit, UmanDiagnostics AB, Umeå, Sweden) was used to measure CSF NFL (pg/L). With the lowest limit of quantification of 50 ng/L, sandwich ELISA is a novel and sensitive method for this measurement (15).

FDG-PET Imaging Processing

The computer package SPM5 (http://www.fil.ion.ucl.ac.uk/spm/) was used to perform the core analyses of 18F-FDG-PET data. We downloaded postprocessed PET images that were coregistered, averaged, and standardized in a voxel size and smoothed to a uniform resolution of 8 mm from the LONI website in NIFTI format. Each image was recentered to correspond to the center of the SPM MNI template and 2 images were generated for each scan of each patient. The ADNI database included 18F-FDG-PET data for the following selected 44 cortical regions of the MNI atlas, which are affected by AD

pathology: bilateral hippocampal (HIPPR and HIPPL), bilateral parahippocampal (PARAHIPR and PARAHIPL), bilateral superior frontal (FRTSUPR and FRTSUPL), bilateral middle frontal (FRTMIDR and FRTMIDL), bilateral inferior frontal (FRTINFR and FRTINFL), bilateral fusiform (FUSFRMR and FUSFRML), bilateral middle occipital (OCCMIDR and OCCMIDL), bilateral angular (ANGULR and ANGULL), bilateral superior parietal (PARSUPR and PARISUPL), bilateral inferior parietal (PARIINFR and PARIINFL), bilateral supramarginal (SUPMRGR and SUPMRGL), bilateral superior temporal (TMPSUPR and TMPSUPL), bilateral middle temporal (TMPMIDR and TMPMIDL), bilateral inferior temporal (TMPINFR and TMPINFL), bilateral precuneal (PRECUNR and PRECUNL), bilateral anterior cingulate (CINGANTR and CINGANTL), bilateral middle cingulate (CINGMIDR and CINGMIDL), bilateral posterior cingulate (CINGPSTR and CINGPSTL), bilateral lingual (LINGUALR and LINGUALL), bilateral insular (INSULR and INSULL), bilateral middle frontoorbital (FRTMIDOR and FRTMIDOL), and bilateral superior middle frontal (FRTSMEDR and FRTSMEDL) gyri. PET scans were acquired at the screening visit and on 6- and 12-month follow-up visits. The baseline CMRGlc value and CMRGlc decline over 6 and 12 months were available in the ADNI. The suffix 01 after regions name corresponds to baseline AD CMRGlc; suffix 02 to baseline MCI CMRGlc; suffix 04 to AD CMRGlc difference over 6-month period corrected for its variance in normal population; suffix 06 to MCI CMRGlc difference over a 6-month period corrected for its variance in normal population; and finally suffix 07 to AD CMRGlc difference over a 12-month period.

SPM5 default settings were used to linearly and nonlinearly deform each baseline PET image to the coordinate space of the SPM brain template. The left-right orientation of each image was confirmed, and therefore spatially normalized to the SPM template space and smoothed. The resulting images were generated in the coordinate space of the SPM template with 2 cubic mm voxel size and $79 \times 95 \times 69$ (in x y z) matrix dimension. The images were further smoothed by a Gaussian kernel with the full width at half maximum of 12 mm in all x, y, and z directions.

To perform the voxel-by-voxel statistical analyses for all cases, the global brain counts were computed by the SPM subroutine spm_global and were normalized using proportional scaling. Appropriate statistics were then calculated on a voxel-by-voxel basis to characterize and compare regional glucose metabolism in the 3 subject groups. Regional CMRGlc in the brain was calculated based on regional tissue time-activity curves for 18F-FDG. This gives a radio-ligand concentration in plasma as a function of time, that is the arterial input function. Briefly, the net influx rate of 18F-FDG from arterial blood samples, steady-state plasma glucose concentration and correction factor for the use of glucose rather than FDG in the brain were used to calculate globally normalized CMRGlc in each region based on a formula proposed previously (16). Energy metabolism in the pons has been conventionally used to normalize measurement of glucose uptake in each voxel as it is reported to be well preserved in AD (17). This is why we used globally normalized values of FDG uptake to the pons in the arterial blood, that is using arterial activity curves to quantify cerebral glucose metabolism, which would give higher diagnostic accuracy in dementia syndromes (18). Moreover, we included a new index, termed hypometabolic convergence index (HCI), derived from a voxel-based image analysis algorithm aimed to quantify similarities in the pattern and scale of hypometabolism to that of a clinically diagnosed patient with AD (19). Therefore, HCI gives a measure of the similarity of a patient's PET results to those in a subject with clinically confirmed AD.

Cognitive Assessment

Cognition was assessed using the Alzheimer's Disease Assessment Scale (ADAS) score, which is a valid test of the degree of cognitive and noncognitive behavioral dysfunctions in patients with dementia (20). ADAS measures of all our participants were available for both 12 questions subscale and a complete total score. Composite scores regarding executive functioning and memory were extracted from the ADNI-I neuropsychological battery (21).

Statistical Analyses

Statistical analyses were performed using SPSS20 software. We investigated potential correlations of the CMRGlc/HCI values of each region of the MNI atlas, with ADAS score, age, sex, plasma NFL and plasma tau, as well as CSF NFL, separately using simple Pearson's correlation model. Next, we implemented a partial correlation model to identify potential confounding covariates to be included in a final regression model. We identified brain regions where CMRGlc/HCI correlated with plasma/CSF NFL or plasma tau, controlling for age, sex, and ADAS score. Finally, we devised a multiple linear regression model to investigate the independent association of each biomarker with CMRGlc of significant regions in the partial correlation analysis in patients with MCI or AD. To test for assumptions of the multiple regression model, we drew the normality Q-Q plots of each variable against the dependent variable for multivariate normality and used the Durbin-Watson test diagnostics for collinearity and the normality plot of residuals to check for the presence of homoscedasticity in variances. The method "Enter" was adopted in multiple linear regression model to identify variables with independent association to the CMRGlc of regions and/or the HCI score. To address the inflation in type I error rate arising due to multiple comparisons, the bootstrap method was used. We examined the reliability of significant regions with type I error rate set at 0.05.

RESULTS

In an exploratory analysis, we investigated potential correlation of CMRGlc values of different brain regions and the HCI index of each patient with plasma tau and plasma and CSF levels of NFL. Our population consisted of 149 subjects, 47 women and 102 men with MCI from the baseline ADNI cohort with a mean age of 75.4 ± 6.58 years. Mean ADAS score of the study group was 19.4 ± 7.7, mean MMSE score was 26.5 ± 3.01, and mean CDR global score was 0.66 ± 0.3, while the mean plasma tau, and mean plasma and CSF NFL

were 2.7 ± 1.3, 42.7 ± 2.54, and 1497.5 ± 757.7 ng/mL, respectively. Investigating bivariate correlations between relevant baseline characteristics, we found a significant negative correlation between patient's age and CMRGlc in the left hippocampus, right parahippocampal regions, right middle, posterior and inferior temporal gyri, left posterior cingulate, right middle occipital gyrus, bilateral middle and superior frontal gyri, right middle frontoorbital gyrus, bilateral anterior cingulate gyri, right insular gyrus, bilateral middle and inferior occipital gyri, as well as right rectus gyrus and parietal gyrus. There was no sex difference between CMRGlc values in most regions, except for a few regions in posterior and middle cingulum, precuneus and inferior temporal cortices. Meanwhile, ADAS score negatively correlated with the CMRGlc in widespread areas throughout the cerebral cortex. With the p value threshold set to 0.1, CMRGlc of all MNI coordinates had an inverse/negative correlation with ADAS score in our study group, in line with our expectation that cerebral hypometabolism was associated with poor cognitive performance in these patients. Based on these preliminary investigations, we adopted the patient's sex, age, and ADAS score as confounding factors in all further analyses on the association between CMRGlc and plasma and CSF markers.

Next, we investigated the association between regional CMRGlc values and plasma NFL (Table 1), CSF NFL (Table 2), and plasma tau (Table 3), respectively. In all these analyses, a negative correlation coefficient of CMRGlc value indicates association of cerebral hypometabolism with higher levels of the biomarker (i.e. inverse correlation), and a positive correlation coefficient of CMRGlc indicates association of cerebral hypermetabolism with higher levels of biomarker (i.e. direct correlation). For this purpose, we first performed a simple bivariate Pearson's correlation between regional CMRGlc and plasma tau, plasma NFL and CSF NFL, and then entered age and sex, and ADAS score as covariates/cofounders to the model where appropriate.

Our findings revealed that lower baseline metabolic activity in the left hippocampus, bilateral parahippocampal, bilateral fusiform, bilateral middle and inferior temporal, and right angular gyri, as well as 6-month decline in metabolic activity of the right hippocampus were all associated with higher plasma NFL levels at the baseline. This association persisted after removing the effect of age and sex (Table 1). With the ADAS score added as a covariate to the model, the negative correlation between plasma NFL and the cerebral metabolic activity persisted only in the bilateral parahippocampal and right fusiform, and the right middle and inferior temporal gyri (Table 1).

CSF NFL level shared almost the same and more widespread correlates to regional CMRGlc as plasma NFL. Higher CSF NFL was associated with lower baseline metabolic activity in the bilateral parahippocampal gyri, right fusiform gyrus, bilateral angular gyrus, left middle and inferior temporal and right superior temporal gyri, bilateral anterior and middle, and left posterior cingulate gyri, bilateral precuneal gyri, left middle occipital gyrus, as well as right middle frontoorbital and left frontal middle-superior gyri. Decline in the cerebral metabolic activity over a 6-month period in the left middle and inferior temporal gyri, and the right superior and posterior cingulate gyri also associated with higher CSF NFL levels at the baseline. Correlation of higher CSF NFL with lower cerebral metabolic rate persisted after correction for age and sex in all mentioned regions, except for left fusiform, rectus and insular gyri (Table 2). After adding ADAS score to the partial correlation model, the 6-month decline in metabolic activity of the posterior cingulate cortex (PCC) and baseline CMRGlc in bilateral precuneal gyri remained significantly correlated to higher baseline CSF NFL levels.

When plasma tau was investigated in a linear model with CMRGlc, lower baseline metabolic activity in the right middle temporal, right middle occipital, right fusiform, right angular, and right inferior parietal gyri, and the left parahippocampal gyrus were associated with higher plasma tau levels at the baseline (Table 3). Higher baseline plasma tau levels also correlated with decline in metabolic activity of glucose in the right middle temporal and right middle occipital gyri over 6 months and decline in metabolic activity of the posterior cingulate gyrus over 12 months. All correlations survived after controlling for the effect of age and sex, but only the correlation of baseline hypometabolism in the left middle occipital

TABLE 1. Significant Results of Pearson's Correlation Analyses of CMRGlc Values and Plasma NFL Levels

Region	Correlation Coefficient†	Corrected for Age and Sex‡	Corrected for Age, Sex, and ADAS Score‡
HIPPL01	−0.243**	−0.188*	−0.088
HIPPR06	−0.205*	−0.213*	−0.231*
PARAHIPL01	−0.300**	−0.268***	−0.171*
PARAHIPR01	−0.360**	−0.314***	−0.225**
FUSFRMR01	−0.343**	−0.356***	−0.278***
FUSFRML01	−0.273**	−0.236**	−0.129
FUSFRML02	−0.236**	−0.191*	−0.072
ANGULR01	−0.169*	−0.249**	−0.137
ANGULR02	−0.165*	−0.256**	−0.152*
ANGULR04	−0.380*	−0.265*	−0.137
TMPMIDR01	−0.171*	−0.283***	−0.175*
TMPMIDR02	−0.238**	−0.302***	−0.219**
TMPMIDL01	−0.187*	−0.226**	−0.104
TMPMIDL02	−0.201*	−0.176*	−0.089
TMPINFR01	−0.368***	−0.283***	−0.193*
TMPINFR04	−0.403	−0.245*	−0.145
TMPINFL04	−0.448	−0.300*	−0.183
TMPPOSR02	−0.163*	−0.094	−0.053
FRTSMEDL02	−0.231**	−0.075	−0.091
FRTMIDOR02	−0.180*	−0.044	0.034
PARIINFR02	−0.200*	−0.241**	−0.135
INSULAR02	−0.218**	−0.118	−0.082
CINGANTL02	−0.198*	−0.080	−0.002
CINGPST07	0.525*	0.510*	0.443
PRECUNR02	−0.172*	−0.156	−0.039

*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.
†Pearson's correlation coefficient of CMRGlc value of the cortical regions and plasma neurofilament protein L (NFL) levels. Negative values indicate correlation of plasma NFL with regional hypometabolism and positive values indicate correlation with regional hypermetabolism.
‡Pearson's correlation coefficient with age and sex and ADAS score.

TABLE 2. Significant Results of Pearson's Correlation Analyses of CMRGlc Values and CSF NFL Levels

Region	Correlation Coefficient†	Corrected for Age and Sex‡	Corrected for Age, Sex, and ADAS Score‡
HIPPL01	−0.180*	−0.145	−0.028
PARAHIPL01	−0.225**	−0.191*	−0.069
PARAHIPR01	−0.244**	−0.216**	−0.103
FUSFRML01	−0.185*	−0.158	−0.024
FUSFRML02	−0.168*	−0.144	−0.003
FUSFRMR01	−0.184*	−0.197*	0.160
ANGULR02	−0.192*	−0.185*	−0.056
ANGULR04	−0.267*	−0.263*	−0.117
ANGULL01	−0.164*	−0.187*	−0.056
ANGULL02	−0.195*	−0.223*	−0.099
TMPSUPR04	−0.237*	−0.270*	−0.210
TMPMIDL01	−0.177*	−0.216**	−0.078
TMPMIDL02	−0.177*	−0.167*	−0.070
TMPMIDL04	−0.248*	−0.247*	−0.187
TMPINFL02	−0.172*	−0.194*	−0.096
TMPINFL04	−0.292*	−0.290*	−0.154
FRTMIDOR02	−0.280**	−0.149*	−0.072
FRTSMEDL02	−0.301**	−0.165*	−0.099
INSULAR02	−0.215**	−0.110	−0.071
RECTUSR02	−0.161*	−0.117	−0.075
SUPMRGL01	−0.201*	−0.223**	−0.121
CINGPSTL01	−0.191*	−0.183*	−0.094
CINGPSTL02	−0.202*	−0.222**	−0.111
CINGPST04	−0.345**	−0.391**	−0.299*
CINGMIDL02	−0.194*	−0.209*	−0.118
CINGANTL02	−0.304**	−0.166*	−0.088
CINGANTR02	−0.248**	−0.095	−0.064
CINGMIDR02	−0.177*	−0.222**	−0.114
PRECUNR01	−0.181*	−0.169*	−0.075
PRECUNR02	−0.229**	−0.270***	−0.159
PRECUNL01	−0.221**	−0.270***	−0.175*
PRECUNL02	−0.215**	−0.273***	−0.181*
OCCMIDL01	0.195*	−0.187*	−0.071
OCCMIDL02	−0.184*	−0.185*	−0.078

*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.
†Pearson's correlation coefficient of CMRGlc value of the cortical regions and CSF neurofilament protein L (NFL) levels. Negative values indicate correlation of CSF NFL with regional hypometabolism and positive values indicate correlation with regional hypermetabolism.
‡Pearson's correlation coefficient with age and sex and ADAS score.

TABLE 3. Significant Results of Pearson's Correlation Analyses of CMRGlc Values and Plasma Tau Levels

Region	Correlation Coefficient†	Corrected for Age and Sex‡	Corrected for Age, Sex, and ADAS Score‡
PARAHIPL01	−0.181*	−0.178*	−0.054
FUSFRMR01	−0.178*	−0.176*	−0.062
ANGULR01	−0.166*	−0.172*	−0.031
TMPMIDR01	−0.165*	−0.176*	−0.059
TMPMIDR02	−0.177*	−0.187*	−0.078
TMPMIDR04	−0.201*	−0.214*	−0.126
PARIINFR01	−0.167*	−0.173*	−0.038
CINGPST07	0.406*	0.497*	0.409
OCCMIDR01	−0.227**	−0.248**	−0.160
OCCMIDR04	−0.210*	−0.221*	−0.115
OCCMIDL01	−0.477*	−0.563**	−0.501*

*$p < 0.05$.
**$p < 0.01$.
†Pearson's correlation coefficient of CMRGlc value of the cortical regions and plasma tau levels. Negative values indicate correlation of plasma tau with regional hypometabolism and positive values indicate correlation with regional hypermetabolism.
‡Pearson's correlation coefficient with age and sex and ADAS score.

gyrus survived in the partial correlation model with age, sex, and ADAS score (Table 3). Finally, HCI score of patients directly correlated with both plasma and CSF levels of NFL (correlation coefficient: 0.230; p value: 0.005, and correlation coefficient: 0.203, p value: 0.013, respectively), meaning that the more similar the patients cerebral hypometabolic patterns were to typical patterns in AD patients, the higher were the baseline plasma and CSF levels of NFL of that patient. This correlation remained significant after correction for age and sex (coefficient: 0.260; p value: 0.001, and correlation coefficient: 0.247, p value: 0.003, respectively).

The above findings indicate that the CMRGlc scores of several cortical regions were correlated with more than one of the investigated plasma or CSF biomarkers, as well as patient's age and sex and the ADAS score in some regions. Therefore, we devised a multiple linear regression model to investigate the independent association of each biomarker with regional CMRGlc in our study group.

In the first step, we chose regions in which CMRGlc was significantly correlated with more than one biomarker, according to our findings in previous analyses. To test for presumptions of the multiple regression model, we investigated multivariate normality by drawing the Q-Q plot of each variable against the dependent variable of interest (i.e. CMRGlc). None of the variables violated the multivariate normality assumption in any of the regions of interest. To check for collinearity, we used the Durbin-Watson test diagnostics, and the normality plot of residuals was used to check for the presence of homoscedasticity in variances. Lastly, we used the method "Enter" in a multiple regression model of variables with potential contribution to the overall variance of CMRGlc value in the following regions: left and right parahippocampal: PARAHIPL01 and PARAHIPR01, left middle temporal: TMPMIDL01, right middle occipital: OCCMIDR01, right fusiform: FUSFRMR01, right angular: ANGULR01, right inferior parietal: PARIINFR01, right middle temporal: TMPMIDR01, left middle temporal: TMPMIDL02, right angular: ANGULR02, right middle temporal: TMPMIDR02, left inferior temporal: TMPINFL04, right angular: ANGULR04, and posterior cingulate: CINGPST07 gyri, as well as the total HCI score of each patient.

Results of the multiple linear regression showed a significant model that positively predicted patients HCI values based on independent contributions of higher CSF NFL levels (p value: 0.003, $F[4,143]$, R^2: 0.328). The regression model

TABLE 4. Significant Results of Multiple Regression Analyses of Regional CMRGlc and Peripheral Biomarkers

Region	Model p Value	R^2*	p Value for Plasma NFL	Corrected Beta Coefficient
PARAHIPL01	0.001	0.336	0.02	−0.214
PARAHIPR01	<0.001	0.372	0.001	−0.319
FUSFMR01	<0.001	0.357	0.001	−0.297
TMPMIDR02	0.032	0.243	0.044	−0.190

CMRGlc, cerebral metabolic rate of glucose; NFL, neurofilament protein L; FUSFRMR01, fusiform right; PARAHIPL01, parahippocampal left; PARAHIPR01, parahippocampal right; TMPMIDR02, temporal mid right.

Suffix 01 after regions name corresponds to baseline AD CMRGlc and suffix 02 to baseline MCI CMRGlc.

*Degree of freedom for the model and residuals for all regions was 3 and 144, respectively.

was also significant for baseline CMRGlc values of bilateral parahippocampal gyri, right middle temporal gyrus, and right fusiform gyrus that were independently and positively predicted by higher plasma NFL levels (Table 4). Neither CSF NFL level, plasma tau level, nor patients' age, sex, and ADAS score could independently predict CMRGlc in any of the study regions.

DISCUSSION

In a cross-sectional study based on the ADNI cohort, we investigated whether plasma or CSF levels of NFL independently predicted regional CMRGlc in patients with MCI. We used a stepwise strategy, starting from investigating potential correlations between plasma and CSF biomarkers and regional CMRGlc values. We narrowed the investigation to areas in which correlations persisted after correction for patient's age and sex, and ADAS score. Finally, we used a multiple linear regression model and identified regions where regional CMRGlc was independently predicted by plasma NFL. When applicable, we used plasma tau, a previously proposed biomarker of progression from MCI to AD, as a covariate in a multiple regression model.

Baseline plasma and CSF NFL levels were both correlated with regional hypometabolism in the screening visit in signature AD regions, including PCC and precuneus from the posteromedial cortex, and the hippocampal and parahippocampal gyri from the medial temporal lobe. Regional decline in CMRGlc of right hippocampus over 6 months and PCC over 12 months of disease progression also correlated with higher baseline plasma NFL and CSF NFL, respectively. Hypometabolism in the parahippocampal, middle temporal, and fusiform gyri was independently predicted by higher plasma CSF levels, and patients HCI by higher CSF NFL levels, in our group of patients with MCI.

Different types of dementia present with distinctive patterns of cortical hypometabolism on PET imaging. Furthermore, there appears to be a stepwise incremental pattern in the number of areas involved. For example, regional hypometabolism in PCC is seen in MCI, whereas hypometabolism is seen in PCC, as well as precuneus, inferior parietal lobule, and middle temporal gyrus in AD patients (22). In our study, hypometabolism in PCC and the medial temporal nodes were associated with higher CSF and plasma NFL levels as well as lower plasma tau. Crucially, these are signature regions within the default mode network (DMN) of the brain that show hypometabolism in early AD.

18F-FDG-PET scan is a highly sensitive and specific technique in detecting neuronal injury. FDG-PET is also highly sensitive in differentiating between various types of dementia with a pooled sensitivity reaching to 91% (23). Meanwhile, 18F-FDG-PET is neither sensitive nor specific in identifying high risk MCI patients who are likely to convert to AD (24). CSF p-tau levels and p-tau/Aβ ratio have been shown to have predictive value for MCI conversion to AD (25) but were later proved to have a higher sensitivity than specificity, and hence a higher negative predictive value to rule out nonconvertors among MCI patients (26). Furthermore, quantification of cortical tau or amyloid burden is yet to achieve gold standard quality to detect high risk MCI patients, as there is a significant overlap in the quantity and distribution of tau and Aβ deposits in MCI, normal aging, and non-AD dementia (27–29). Plasma and CSF levels of NFL are increased in MCI and associated with clinical severity and progression to AD (7, 30, 31). CSF NFL levels are even elevated in carriers of the *APOEε4* gene, who have a higher risk of developing AD (32), and increase with progression from MCI to AD (33). We hypothesized that NFL could be used as a clinically relevant marker of cerebral hypometabolism in high risk patients with MCI.

The DMN is a major functional network of the brain involved in several semantic and cognitive functions, including memory processing, encoding and recall. The medial temporal hub, consisting of the medial temporal lobe, and the core hub are main functional hubs within the DMN (34). The parahippocampal gyrus of the medial temporal hub works in concert with the PCC and retrosplenial cortex from posteromedial cortex, as activation of the former and deactivation of the latter is essential for successful memory encoding (35, 36). Our findings revealed that higher plasma and CSF NFL levels correlated to hypometabolism in cortical areas within the core hub of DMN, consisting of the PCC, precuneal gyrus, and parts of the retrosplenial cortex (34, 37), and within the medial temporal hub of the DMN, including the parahippocampal gyrus, the hippocampus, and the temporal poles. Importantly, we found that higher CSF NFL levels were associated with hypometabolism in the medial temporal nodes as well as the PCC and precuneal gyrus from the posteromedial cortex, whereas plasma NFL levels correlated only with MTL medial temporal nodes, suggesting a higher sensitivity for CSF NFL compared to plasma NFL levels.

CSF NFL level was also correlated to hypometabolism in the medial frontal and angular gyri which are not among signature areas in AD. Importantly, a paradoxical increase in the metabolic activity of the hippocampal cortex is observed in patients with MCI (38). This is believed to be a rebound hyperactivity due to loss of global functional connections of the medial temporal lobe, in particular those attributing to the frontal cortical areas (39). This might explain the association of frontal cortical hypometabolism with high CSF NFL in MCI patients. Importantly, the medial frontal cortex shows relatively higher amyloid beta deposition and low hypometabolism in MCI compared to significant hypometabolism and atrophy seen in signature AD regions within the medial temporal lobe (40). Therefore, correlation of CSF NFL levels with hypometabolism in extra-DMN neocortical regions, including the medial frontal and angular gyri, has a predictive potential for conversion from MCI to AD (40).

Importantly, our results suggest that plasma tau and plasma NFL levels correlate with cortical hypermetabolism in the PCC region. Association of plasma tau and NFL with PCC hypermetabolism can be justified in the light of the following findings regarding the role of amyloid beta in cortical metabolic activity: first; Aβ deposition is shown to be incongruently high in the PCC of patients with AD dementia, despite minimal degree of atrophy (41), and second; posterior associational cortices, including the PCC, which show Aβ-associated hypermetabolism in early AD, are shown to be more vulnerable to hypometabolism and atrophy in later disease stages (42). To conclude, Aβ-associated hypermetabolism in the PCC correlated with higher plasma NFL and tau levels in our sample, suggesting a predictive role for plasma tau in high risk MCI patients.

As expected, and in line with previous studies (7), CSF NFL and plasma NFL levels were correlated in our sample (correlation coefficient: 0.448, $p < 0.001$). Importantly, most of the CSF NFL: CMRGlc correlates did not survive correction for ADAS score, and CSF NFL did not show independent association with regional hypometabolism in the multiple regression model. Moreover, since the blood-derived biomarkers are less invasive and costly to measure, and considering the promising results regarding plasma NFL to independently predict CMRGlc in AD signature regions, plasma NFL can be considered as an early biomarker of to identify high risk MCI patients.

Conclusion

Early detection and follow-up of progression of dementia is important. A remarkable advantage of using blood-based biomarkers compared to CSF markers or imaging techniques is a great reduction in follow-up expenses. It is to be hoped that plasma NFL is used as a noninvasive, inexpensive biomarker for conversion of MCI into AD, and perhaps for AD progression.

ACKNOWLEDGMENTS

Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Cogstate; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

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