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CSF neurofilament concentration reflects disease severity in frontotemporal degeneration

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Abstract

Objective—Cerebrospinal fluid (CSF) neurofilament light chain (NfL) concentration is elevated in neurological disorders including frontotemporal degeneration (FTD). We investigated the clinical correlates of elevated CSF NfL levels in FTD.

Methods—CSF NfL, amyloid-β_{42} (Aβ42), tau and phosphorylated tau (ptau) concentrations were compared in 47 normal controls (NC), 8 asymptomatic gene carriers (NC2) of FTD-causing mutations, 79 FTD (45 behavioral variant frontotemporal dementia [bvFTD], 18 progressive nonfluent aphasia [PNFA], 16 semantic dementia [SD]), 22 progressive supranuclear palsy, 50 Alzheimer's disease, 6 Parkinson's disease and 17 corticobasal syndrome patients. Correlations between CSF analyte levels were performed with neuropsychological measures and the Clinical Dementia Rating scale sum of boxes (CDRsb). Voxel-based morphometry of structural MR images determined the relationship between brain volume and CSF NfL.

Results—Mean CSF NfL concentrations were higher in bvFTD, SD and PNFA than other groups. NfL in NC2 was similar to NC. CSF NfL, but not other CSF measures, correlated with CDRsb and neuropsychological measures in FTD, and not in other diagnostic groups. Analyses in two independent FTD cohorts and a group of autopsy verified or biomarker enriched cases confirmed the larger group analysis. In FTD, gray and white matter volume negatively correlated with CSF NfL concentration, such that individuals with highest NfL levels exhibited the most atrophy.

Interpretation—CSF NfL is elevated in symptomatic FTD and correlates with disease severity. This measurement may be a useful surrogate endpoint of disease severity in FTD clinical trials. Longitudinal studies of CSF NfL in FTD are warranted.

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Introduction

Frontotemporal degeneration (FTD) is a common form of dementia in individuals with disease onset prior to 65 years of age.[1] FTD encompasses three main clinical syndromes, a behavioral variant (bvFTD) and two primary aphasias, a progressive nonfluent variant (PNFA) and a semantic dementia variant (SD).[1,2] FTD is pathologically distinct from Alzheimer's disease (AD), with most cases displaying either insoluble deposits of tau protein or TAR DNA binding protein 43kDa (TDP-43) in neurons and glia at autopsy, whereas in AD is associated with deposits of tau in the form of neurofibrillary tangles as well as plaques containing β amyloid$_{1–42}$ (Aβ42).

CSF Aβ, tau and phosphorylated tau (ptau) at residue 181 are commonly used diagnostic biomarkers for AD, and have been used as surrogate endpoints in clinical trials of disease modifying agents for AD.[3] Elevations in CSF tau and ptau are thought to represent neuronal degeneration while decreases in CSF Aβ42 likely reflect plaque deposition.[3,4] In contrast to AD, CSF Aβ42 and tau are not consistently altered in FTD. Some studies have demonstrated modest tau elevations in FTD while others report normal levels.[5,6,7,8] These disparate results could reflect the pathological heterogeneity in clinically-diagnosed FTD.[9] Aβ42 levels in FTD are comparable to normal controls (NC).[5,6,7,8,10]

Neurofilaments are structural components of axons and are measurable in CSF.[11,15] Increased CSF concentrations of neurofilament proteins, including neurofilament light chain (NfL) and phosphorylated neurofilament heavy chain (pNfH), have been associated with neuronal death and axonal degeneration in a variety of disorders including AD,[12,] Parkinson's disease,[14] multiple sclerosis,[15,16] and amyotrophic lateral sclerosis (ALS).[12,15] In MS [17] and ALS,[18] CSF NfL concentration is correlated with disease severity. Elevated CSF NfL has previously been reported in FTD, however it is not known whether different FTD subtypes or clinical features are associated with elevated CSF NfL.[12,19]

The goals of this study were therefore to: 1) examine CSF NfL levels in different FTD clinical syndromes, 2) compare NfL to CSF biomarkers associated with AD (including Aβ42, tau, and ptau) in each of these syndromes, and 3) determine if CSF NfL levels relate to clinical or neuroimaging measures of FTD.

Methods

Subjects

229 individuals were evaluated at the UCSF Memory and Aging Center. 79 subjects met Neary[1] criteria for FTD: 45 bvFTD, 18 PNFA and 16 SD. Other clinical neurodegenerative groups met established diagnostic criteria, including 50 NINCDS-ADRDA probable AD,[20] 22 NINDS-SPSP probable or possible progressive supranuclear palsy (PSP),[21] 6 Parkinson's disease (PD),[22] and 17 corticobasal syndrome (CBS).[23] 47 normal controls (NC) had normal neurological examinations, neuropsychological testing scores and clinical dementia rating (CDR) scores of 0 (27 were evaluated at UCSF and 20 were samples purchased from Precision Med [San Diego, CA]). Eight individuals were asymptomatic carriers (NC2) of known FTD causing mutations (C9 open reading frame 72 hexanucleotide repeat expansion

[*C9ORF72*] [24], progranulin [*GRN*] [25] or tau [*MAPT*])[26]. Study participants provided written informed consent, and all procedures were approved by the UCSF IRB.

Biomarker enriched cases

Since FTD syndromes can sometimes be caused by atypical AD pathology, analyses were repeated in a subset of individuals who had either 1) a known FTD-causing mutation, 2) had been previously characterized with the amyloid PET agent, Pittsburgh Compound B (PiB),[27] 3) or had an autopsy confirmed frontotemporal lobar degeneration (FTLD) diagnosis. In total, 76 subjects were gene carriers, had PiB data or autopsy confirmed diagnoses (Supplemental Methods and Supplemental Table 1).

Neuropsychological testing

General cognition was assessed by the Mini-mental state examination (MMSE).[28] Visuospatial abilities were examined through copy of a modified Rey-Osterrieth figure (Rey).[29] Working memory was assessed using forward and backward digit span (FDS and BDS).[30] Executive functioning was assessed using a modified Trail-making test (Trails),[31] and the Stroop task (number correct for both color and interference condition).[32] Language was assessed using a 15-item Boston naming task (BNT)[33] and a phonemic fluency (D words per minute) and category fluency task (animals per minute). Verbal short term memory was assessed by a nine item California verbal learning task (CVLT) [34] and visuospatial memory was assessed by 10 minute recall of the modified Rey figure. The clinical dementia rating (CDR; including sum of boxes [CDRsb]) assessed disease severity.[35]

CSF Analyses

LPs were performed using the ADNI protocol (http://www.adni-info.org/ADNIStudyProcedures/LumbarPunctures.aspx). The INNO-BIA AlzBio3 (Innogenetics, Ghent, Belgium) platform was used to measure Aβ42, tau and ptau. CSF NfL levels were measured using the Uman Diagnostics ELISA kit (Umea, Sweden; Supplemental Methods). NfL ELISA's were run on 7/26/2012 and 4/24/2013. Samples analyzed in the first experiment comprised the *original* cohort and were from 29 NC, 22 bvFTD, 10 SD, 8 PNFA, 31 AD, 11 PSP and 9 CBS patients evaluated between 2/19/2009 – 2/9/2012. A second set of samples from different patients (17 NC, 22 bvFTD, 6 SD, 10 PNFA, 17 AD, 10 PSP and 7 CBS) evaluated between 7/1/2009 – 3/4/2013 comprised a *validation* cohort. A *combined* cohort, consisting of all samples re-analyzed in the same ELISA (4/24/2013) was used to increase the sample size for the neuropsychological and imaging correlation analyses.

Statistics

Data were analyzed using SPSS (version 16.0, SPSS Inc., Chicago). Normality for individual variables was determined by the Shapiro-Wilk test. Mean values were compared using univariate analyses of variance, with Tukey post-hoc analyses for normally distributed samples. Non-parametric tests, Kruskal-Wallis H and Mann-Whitney U, were used to compare group values for data that was not normally distributed (Supplemental Methods).

Relationships between CSF analytes, cognitive and demographic data were examined using Pearson or Spearman correlations. A threshold of $p < 0.05$ corrected for multiple comparisons (false discovery rate [36]) was accepted as significant.

MR Imaging

A total of 66 FTD (39 bvFTD, 13 SD, 14 PNFA) had structural MRI data. Brain images were acquired using a 3T Siemens Tim Trio scanner equipped with a 12-channel receiver head coil. T1-weighted images of the entire brain (TR/TE/TI = 2300/2.98/900 ms, flip angle of 9°, a bandwidth of 240 Hz/pixel, sagittal orientation with a FOV = 256 × 240 mm and 160 slices, voxel size = 1 mm^3).

Voxel-based morphometry (VBM)

Statistical Parametric Mapping 8 (SPM8; http://www.fil.ion.ucl.ac.uk/spm) was used to analyze the MRI data. Using the VBM8 toolbox (http://dbm.neuro.uni-ena.de/vbm.html), segmented grey and white matter volumes were created. Volumes were smoothed with a 12-mm FWHM Gaussian filter. Whole-brain Spearman correlations were performed to investigate the relationship between individual NfL concentrations and both gray and white matter atrophy.

Results

Demographics and neuropsychological performance

There were no differences between the original and the validation cohorts in age, disease duration, education, or neuropsychological performance (Supplemental Table 1). In the combined cohort, asymptomatic gene carriers (NC2) were younger than NC, PNFA, AD, PSP, PD and CBS ($p < 0.026$; Table 1) and bvFTD patients were younger than PNFA ($p = 0.006$). There were no other differences in gender, education or disease duration between patient groups. CDRsb scores were higher in all patient groups compared to NC and NC2 ($p < 0.025$). Neuropsychological test scores were lower in most patient groups as compared to NC. In particular, MMSE scores were lower in all patient groups except PD than NC ($p < 0.001$) and were lower in PNFA and AD compared to NC2 ($p < 0.05$).

Group differences in CSF biomarkers (Figure 1)

CSF NfL—In the original cohort, CSF NfL levels were higher in all FTD subgroups (bvFTD, SD and PNFA) than NC ($p < 0.001$) and AD ($p < 0.03$). bvFTD and SD also had higher NfL concentrations than PSP ($p < 0.003$). NfL levels were also higher in AD, PSP and CBS compared to NC ($p < 0.001$). To confirm these results, we ran a second NfL ELISA on CSF samples collected from a new group of patients. In this *validation* cohort, CSF NfL levels were also higher in all FTD subgroups than NC ($p < 0.001$). SD and PNFA, but not bvFTD ($p = 0.795$), also had higher NfL concentration than AD ($p < 0.006$) and PSP ($p < 0.007$). NfL concentrations were also higher in AD and PSP compared to NC ($p < 0.001$). In the combined cohort, CSF NfL concentrations were higher in all FTD subgroups (bvFTD, SD and PNFA) than NC ($p < 0.001$), AD ($p < 0.006$), NC2 ($p < 0.009$) and PD ($p < 0.006$). SD and PNFA also had higher NfL concentrations than PSP ($p < 0.037$). NfL concentrations were also higher in AD compared to NC ($p = 0.001$). NfL levels in NC2 were similar to NC.

CSF Aβ, tau and ptau—In the combined cohort, AD patients had lower CSF Aβ42 concentrations than all other groups ($p < 0.001$) except PSP and CBS. CSF tau concentrations were higher in bvFTD, SD, PNFA and AD than NC ($p < 0.036$) and AD had higher tau concentrations compared to PSP and PD ($p < 0.03$). CSF ptau concentrations were higher in AD than NC, bvFTD, SD, PNFA and PSP ($p < 0.012$). AD patients had higher tau/Aβ42 ratios than all other groups ($p < 0.007$). ptau/Aβ42 ratios were also higher in the AD group than all other groups ($p < 0.001$).

Correlations between CSF analytes, clinical and neuropsychological ratings

NfL and disease severity—In the original cohort there was a positive correlation between CSF NfL and CSRsb in all FTD combined ($\rho = 0.413$, $p = 0.008$) and a negative correlation with MMSE scores ($\rho = -0.332$, $p = 0.039$). These findings were replicated in the validation cohort, with a positive correlation between CSF NfL concentration and CDRsb in all FTD ($\rho = 0.359$, $p = 0.052$) and a negative correlation with MMSE ($\rho = -0.549$, $p = 0.002$). To increase power to detect correlations with neuropsychological variables in the different FTD subgroups (bvFTD, SD and PNFA), we used the combined dataset to investigate the specificity of the clinical-NfL correlations. There was a positive correlation between CSF NfL concentration and CDRsb in bvFTD ($\rho = 0.406$, $p = 0.008$), SD ($\rho = 0.638$, $p=0.019$) and PNFA ($\rho = 0.632$, $p=0.011$). There was no relationship between CSF NfL levels and disease severity in any other diagnostic group. There also was no relationship between CDRsb and Aβ42, tau or ptau levels in any group (Supplemental Figure 1).

NfL and neuropsychological performance—In the original cohort NfL levels negatively correlated with BDS ($\rho = -0.474$, $p = 0.005$), phonemic fluency ($\rho = -0.535$, $p = 0.002$), category fluency $\rho = -0.564$, $p = 0.001$), Stroop color naming ($\rho = -0.409$, $p = 0.038$) and interference ($\rho = -0.485$, $p = 0.016$). In the validation cohort, NfL levels negatively also correlated with phonemic fluency ($\rho = -0.440$, $p = 0.019$), category fluency ($\rho = -0.648$, $p = 0.001$), BNT ($\rho = -0.403$, $p = 0.022$), Stroop color naming ($\rho = -0.527$, $p = 0.012$) and Stroop interference ($\rho = -0.547$, $p = 0.010$), CVLT 30 second recall ($\rho = -0.560$, $p = 0.004$) and CVLT 10 minute recall ($\rho = -0.412$, $p = 0.045$). CSF NfL was also correlated with neuropsychological performance in bvFTD and PNFA, individually (Supplemental Data).

Confirmation in biomarker enriched cases—We repeated the CSF analyses in the subgroup of 44 FTD subjects who had increased likelihood of FTD pathology based on a known FTD-causative mutation, low fibrillar amyloid levels as measured by PiB PET, or an autopsy confirmed FTLD diagnosis, as compared to 14 PiB+ or autopsy-confirmed AD patients (Supplemental Table 2). Consistent with the results in the larger group, NfL levels were higher in the biomarker enriched FTD cases as compared to AD ($p = 0.001$). There was also a negative correlation between CSF NfL levels and MMSE in the biomarker enriched FTD group ($\rho = -0.376$, $p = 0.018$).

CSF NfL and brain atrophy—Since CSF NfL levels were correlated with disease severity in FTD, we hypothesized that NfL would also be correlated with brain volume in regions associated with disease in a subgroup of the combined FTD cohort who had high quality MRI data. Using a non-parametric approach in VBM, we identified negative

correlations between CSF NfL concentration and gray matter density in all FTD patients ($\rho < -0.353$, $p < 0.05$ FDR corrected, Figure 3A; Supplemental Table 3) and bvFTD alone ($\rho < -0.441$, $p < 0.05$, Figure 3B). In both groups, brain atrophy was mostly left lateralized. In All FTD patients, CSF NfL correlated with gray matter volume in frontal, temporal, parietal, occipital and cingulate cortices, with similar correlations observed in bvFTD only. Less prominent correlations were identified in the white matter associated with most of these regions (Supplemental Table 3).

Discussion

We found that CSF NfL concentrations were elevated in all three FTD clinical subtypes, bvFTD, SD and PNFA, as compared to healthy controls and other neurodegenerative diseases such as AD, PD and PSP. Importantly, CSF NfL levels reflected disease severity as measured by CDRsb in FTD, but there were no such relationships identified with standard AD CSF biomarkers, and no correlations were identified between CSF NfL and disease severity in other disorders. These findings were replicated in an independent cohort of FTD patients and controls. Further supporting the relationship between CSF NfL and disease severity in FTD, clinically normal, asymptomatic carriers of FTD causing mutations (NC2) had CSF NfL concentrations similar to NC. Consistent with the correlation between CSF NfL and disease severity, NfL levels were also strongly related to gray and white matter volume in FTD, with higher CSF NfL indicating more atrophy. Together, these findings suggest that CSF NfL may be a useful biomarker of disease severity in FTD.[37]

The elevated CSF NfL concentrations we observed in FTD are consistent with previous reports. CSF NfL levels have been suggested to indicate neuronal death and axonal degeneration in neurological disease.[14,19] Previous studies have found increased CSF NfL in FTD compared to healthy controls, however variable differences between FTD and AD have been reported. While some studies showed elevated levels in FTD compared to AD[38,39] others did not reveal group differences.[46] Since clinical FTD may be caused by underlying tau, TDP-43 or other rare forms of neuropathology, as well as atypical presentations of AD, these disparate results could result from differences in the neuropathological composition of the cohorts that were studied.[13] To help exclude this potential confound, we examined CSF results in FTD cases that were PiB-, had known genetic causes of FTD, or were autopsy confirmed, and found comparable associations between NfL levels, disease status and severity.

Mean CSF NfL concentrations were similarly elevated in all three FTD clinical syndromes, however the distribution of values in each group was different. While overall, the highest NfL levels were found in bvFTD patients, there was a wide range of values within this clinical subtype, including some individuals with low CSF NfL concentrations. In contrast, the SD group had a much narrower range of CSF NfL concentrations, with no individuals displaying CSF NfL concentrations in the NC range. This result is similar to another recent study of CSF NfL that demonstrated similar elevations in CSF NfL in SD.[39] Together, these data suggest that CSF NfL may be more prominently elevated in individuals with SD than other FTD subgroups. Whether these elevations reflect the association of SD with FTLD-TDP pathology,[40] or some other component of SD biology will require further study in a

larger cohort of autopsy-confirmed cases. The finding that NfL was elevated in PNFA, a disorder most commonly associated with tau pathology argues against a specific association with FTLD-TDP.

We found that CSF NfL concentrations in FTD were also correlated with decreased gray and white matter volume in FTD-associated regions in the frontal and temporal lobes. The frontal lobe volume correlations with NfL concentration are consistent with previous reports of regional brain atrophy correlated with disease severity. [37,48] In addition, in bvFTD, parietal lobe volume was also correlated with CSF NfL concentration. Parietal lobe atrophy has been previously identified in bvFTD patients, usually later in the course of disease, and may be more common in certain FTLD-TDP associated diagnoses such as FTD/ALS associated with *C9ORF72*.[48,41,42] Because NfL is an axonal protein, we hypothesized that there would also be correlations between CSF NfL and regional white matter volumes. Decreased white matter volumes were identified in association with elevated CSF NfL levels in a number of regions bordering (or overlapping—an artifact of the 12 mm smoothing kernel used in the VBM analysis[43]) many of the regions reported in found in the gray matter analysis. Future studies applying more sensitive white matter measurements, such as diffusion tensor imaging may better elucidate the relationship between white matter integrity and CSF NfL in FTD.

Our results with standard AD CSF biomarkers (tau, ptau and Aβ42) in FTD are similar to those reported in the literature. Some previous studies have shown elevated CSF tau levels,[3, 5,9,42] whereas others revealed normal tau levels.[4,5,6,7,8] We found elevated total tau in FTD and AD as compared to NC, but ptau was only elevated in AD, similar to what has been reported previously.[9] The CSF findings in AD are consistent with previous studies [44,44] suggesting that our CSF assays behaved similarly to those used by other investigators.

There are important limitations to this study. We evaluated only NfL in CSF, and did not measure other neurofilament biomarkers, such as pNfH, which may also be a sensitive biomarker of FTD.[13] Also, most of our diagnoses were clinically determined, with only a small subset of autopsy-confirmed diagnoses; therefore it was not possible to know the molecular pathology associated with many of the FTD cases we studied. Although we replicated most of the NfL concentration differences between different cohorts in two separate cohorts, we were not able to replicate the difference in NfL concentration between bvFTD and AD in the validation cohort, suggesting that CSF NfL may not be valuable for differentiating these two clinical syndromes.

In summary, CSF NfL concentrations were strikingly elevated in FTD, particularly in cases associated with FTLD-TDP pathology. Since CSF NfL was correlated with disease severity and brain atrophy, our findings suggest that CSF NfL might eventually be used as a surrogate outcome measure in future clinical trials of disease modifying agents for FTD.[45] Further studies in longitudinal FTD cohorts are warranted to fully establish CSF NfL as a biomarker of disease severity in FTD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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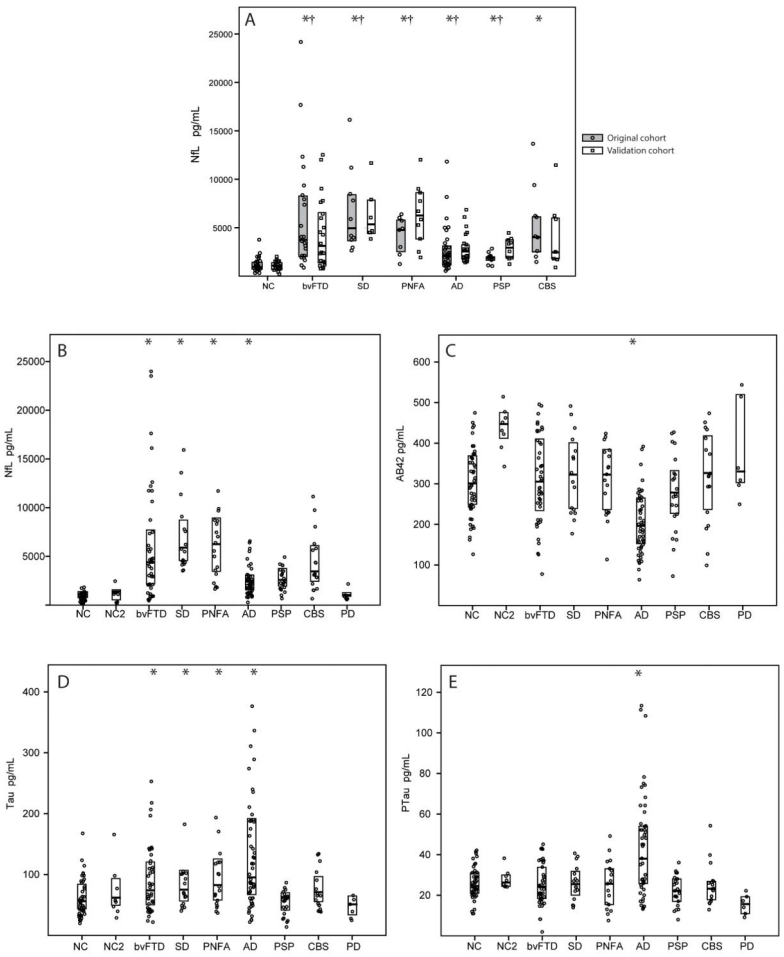

Figure 1. Group comparisons of CSF analyte concentrations

A) Individual values for CSF NfL concentration for the the original cohort (circles, shaded boxes) compared to the validation cohort (squares, open boxes), investigating healthy normal controls (NC) to all patient groups. * and † indicates differences between NC and patient groups for original ($p< 0.001$) and validation cohorts ($p< 0.001$), respectively. B)–E) Individual values for CSF concentrations of B) NfL (combined cohort), C) Aβ, D) Tau, E) ptau, comparing NC to all patient groups. Univariate Analysis of Variance or Kruskal-Wallis tests were used to determine group differences (see Methods). * indicates differences between NC and patient groups: B) bvFTD, SD, PNFA and AD, $p< 0.001$; C) AD, $p< 0.001$; D) bvFTD, SD, PNFA and AD, $p< 0.036$; E) AD, $p< 0.001$.

For the boxplots, the middle line indicates median; bottom and top of box indicate the 25^{th} and 75^{th} percentile, respectively. Abbreviations: NC= normal controls, NC2= clinically normal carriers of known FTD-causing mutations, bvFTD= behavioral variant FTD, SD = semantic dementia, PNFA = progressive non-fluent aphasia, AD= Alzheimer's disease, PSP= progressive supranuclear palsy, CBS= Corticobasal syndrome, PD= Parkinson's disease.

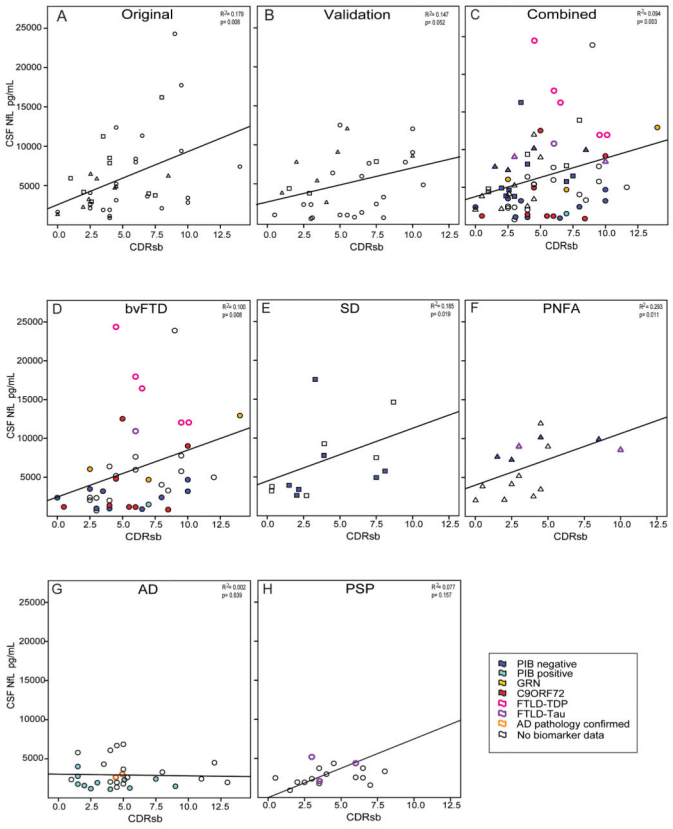

Figure 2. CSF Neurofilament concentrations and disease severity in FTD, AD and PSP

Correlations between NfL and disease severity as measured by CDRsb in all FTD subtypes: A) original cohort, B) validation cohort, and C) combined cohort. Symbols: ● = bvFTD, ■ = SD and ▲ = PNFA. ii) Correlations between NfL and CDRsb in disease subtypes: D) bvFTD; E) SD; F) PNFA; G) AD; H) PSP. Colored and filled symbols indicate cases with additional autopsy, genetic or PiB data used in confirmatory analysis, from biomarker enriched cohort.

Abbreviations: CDRsb: Clinical dementia rating sum of boxes, NC= normal controls, bvFTD= behavioral variant FTD, SD = semantic dementia, PNFA = progressive non-fluent aphasia, all FTD subtypes: bvFTD+SD+PNFA, AD= Alzheimer's disease, PSP= progressive supranuclear palsy, CBS= Corticobasal syndrome, PD= Parkinson's disease

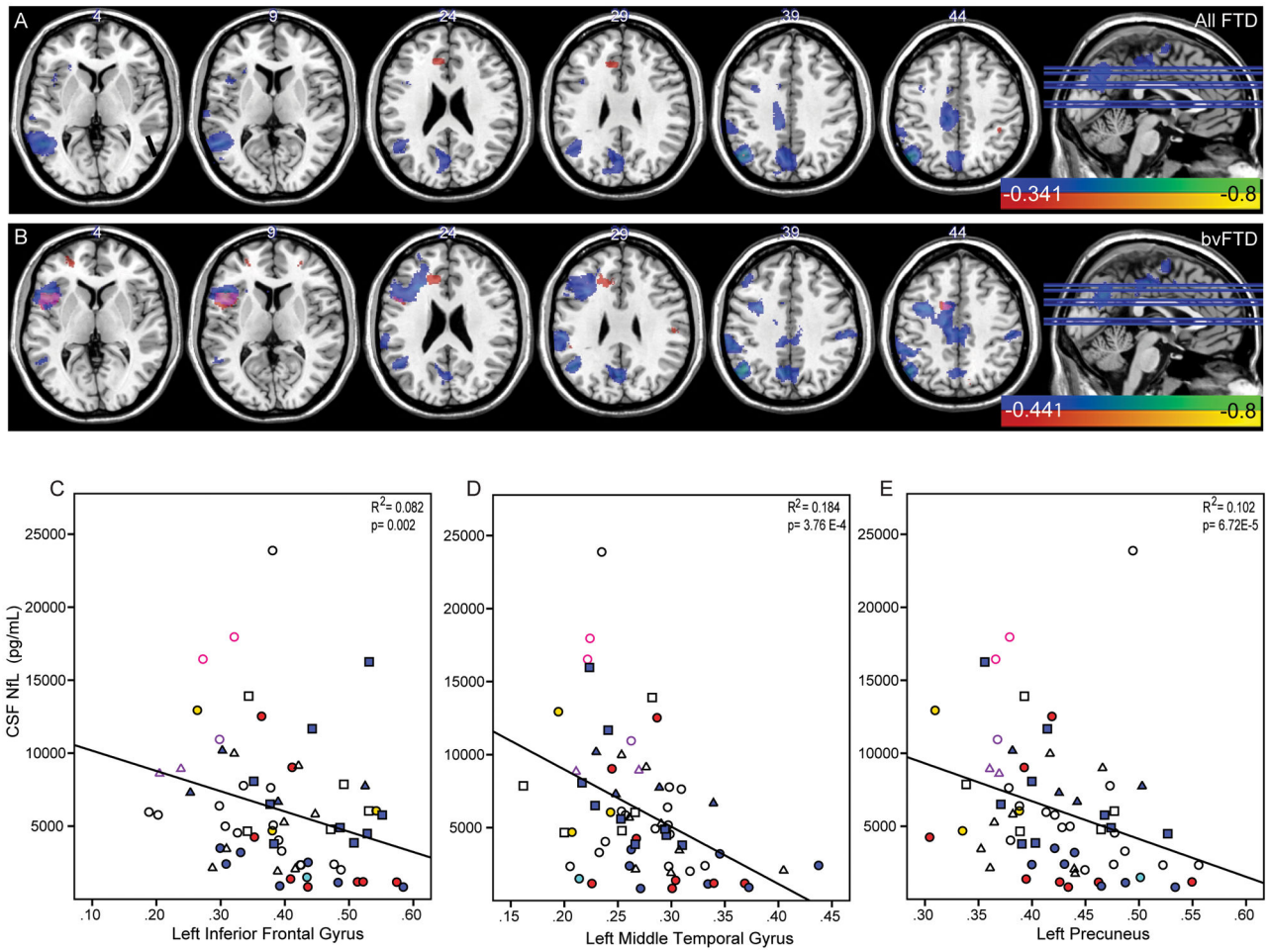

Figure 3. Regional brain volume correlates with CSF NfL concentration in FTD

A), B): Negative correlations between CSF NfL and gray (blue) and white (red) matter volume in lateralized frontal, temporal and parietal regions, with overlapping clusters in pink. Overlap occurs due to 12 mm smoothing kernel used for VBM analysis (see Methods). Shown in: A) All FTD patients (n=66), and B) only bvFTD patients (n=39). Spearman analyses, 2-tailed, $p_{uncorrected} < 0.005$ for display purposes. FDR-corrected statistics for each region given in Supplemental Table 3.

C), D), E): Scatterplots of CSF NfL concentrations versus individual subjects' signal intensity at peak voxels in selected gray matter clusters. Regions selected are peak voxels from the Spearman correlational analysis, correlated ($p < 0.05$, False Discovery Rate corrected) in the all FTD image. Montreal Neurological Coordinates: C) left inferior frontal gyrus [−36, 13, 15], D) left middle temporal gyrus [−53, −53, 4], E) left precuneus [−8, −64, 23]. Please refer to Figure 2 for symbol and color legends.

Table 1

Demographics and Neuropsychological Testing

B) Combined cohorts	NC	NC2	FTD			AD	PSP	CBS	PD	Statistical analysis	Post-hoc analysis
			bvFTD	SD	PNFA						
Demographics											
n	47	8	45	16	18	50	22	17	6		
Age	66(11)	54(10)	61(8)	63(7)	70(7)	66(9)	68(7)	68(8)	70(5)	F(8,229)= 4.54	NC2 > NC, PNFA, AD, PSP, PD, CBS PNFA > bvFTD
Gender (M/F)	26/21	4/4	32/13	6/10	11/7	28/22	11/11	6/11	3/3	$\chi^2(8,229)= 10.02$	ns
Education (years)	17(2)	20(3)	16(3)	17(2)	17(4)	15(4)	15(2)	15(3)	17(3)	F(8,180)= 2.17	ns
Duration (years)	N/A	N/A	6.5(5.8)	4.4(1.6)	5.5(2.3)	5.1(2.1)	6.4(2.3)	4.8(3.9)	N/A	F(5,81)= 0.690	ns
General											
MMSE	29.0(1.8)	28.3(0.6)	24.6(2.6)	21.5(8.4)	23.6(4.8)	19.9(5.8)	26.2(3.3)	25.5(3.2)	28.5(0.8)	H(8)= 91.22	NC > ALL NC2 >AD, PNFA AD < bvFTD, PNFA, PSP, PD, CBS PNFA< PSP, PD
CDR-sb	0.0(0)	0.0(0)	6.03(3.1)	4.1(2.5)	3.7(2.7)	4.9(3.1)	4.2(2.1)	3.1(2.5)	0.7(0.9)	H(8)= 85.63	NC & NC2 < ALL bvFTD > AD, CBS PD < bvFTD, SD, PNFA, AD
Memory											
Mod. Rey recall (max. 17)	12.2(3.4)	14.0(0)	6.9(5.1)	7.7(5.1)	8.2(4.3)	4.7(4.1)	9.3(2.7)	9.3(5.2)	12.0(1.3)	F(8,127)= 5.28	NC > bvFTD, AD
CVLT 10 min recall (max. 9)	7.9(1.3)	8.0(1.4)	3.7(2.9)	2.0(2.2)	3.7(3.2)	2.5(2.5)	6.1(1.5)	6.0(3.4)	7.2(0.8)	F(8,132)= 9.32	NC > bvFTD, SD, PNFA, AD PSP > SD, AD
Language											
BNT (max. 15)	14.7(0.5)	14.5(0.7)	12.8(3.2)	4.1(3.9)	10.7(4.6)	11.2(3.7)	14.3(0.9)	12.3(3.2)	14.5(1.2)	F(8,152)= 14.94	NC > PNFA, AD SD < ALL
D-words / min	16.2(4.0)	20.5(0.7)	8.3(5.3)	8.3(4.4)	5.7(3.8)	8.0(4.3)	6.2(3.5)	7.4(3.8)	12.5(5.2)	F(8,146)= 11.50	NC & NC2 > bvFTD, SD, PNFA, AD, PSP, CBS
Animals / min	24.3(4.6)	27.5(2.1)	12.0(6.0)	6.2(5.2)	8.3(6.1)	9.7(5.5)	10.4(3.3)	10.4(5.7)	18.5(10.9)	F(8,152)= 19.92	NC & NC2 > bvFTD, SD, PNFA, AD, PSP, CBS

B) Combined cohorts	NC	NC2	FTD			AD	PSP	CBS	PD	Statistical analysis	Post-hoc analysis
			bvFTD	SD	PNFA						
CVLT 30 sec (max. 9)	8.2(0.8)	8.5(0.7)	5.1(2.6)	3.3(2.8)	4.5(3.2)	3.5(2.1)	7.2(1.3)	6.3(2.3)	7.7(1.0)	F(8,132)= 8.29	NC > bvFTD, SD, PNFA, AD SD < PSP, PD, CBS
Visuospatial											
Mod. Rey copy (max. 17)	15.7(0.6)	16.0(0)	14.7(1.6)	15.9(0.7)	14.6(2.1)	12.0(4.6)	12.8(1.7)	13.1(2.4)	16.0(0.9)	F(8,128)= 5.08	AD < NC, bvFTD, SD, PD
Executive											
Digit forward	7.3(0.9)	8(1.4)	5.8(1.4)	6.3(1.7)	4.9(1.4)	4.7(4.1)	5.5(1.4)	5.0(1.0)	6.2(1.5)	F(8,153)= 7.67	NC > bvFTD, PNFA, AD, PSP, CBS NC2 > AD
Digit backward	5.9(1.3)	5.5(2.1)	5.8(1.6)	4.6(1.1)	3.3(1.4)	2.9(1.0)	3.5(0.9)	3.2(1.3)	5.0(1.1)	F(8,152)= 11.95	NC > bvFTD, PNFA, AD, CBS
Trails (lines/min)	0.6(0.3)	0.6(0.2)	5.6(3.6)	0.4(0.2)	0.2(0.1)	0.2(0.2)	0.2(0.1)	0.2(0.2)	0.5(0.3)	F(8,135)= 11.06	NC < bvFTD, PNFA, AD, PSP, CBS
Stroop Color (n correct)	93.8(14.6)	91.0(0)	54.3(24.3)	72.3(29.9)	36.3(14.7)	44.8(22.3)	49.3(18.5)	34.9(18.1)	79.0(15.2)	F(8,120)= 13.02	NC > bvFTD, PNFA, AD<PSP, CBS NC2 > PNFA, CBS SD > PNFA, AD, CBS
Stroop Interference (n correct)	59.3(9.9)	60.5(14.9)	27.9(13.7)	43.8(16.6)	19.9(6.8)	16.4(13.3)	23.8(7.5)	18.7(12.1)	46.0(6.2)	F(8,115)= 24.15	NC > bvFTD, PNFA, AD<PSP, CBS NC2 > bvFTD, PNFA, AD<PSP, CBS SD > bvFTD, PNFA, AD<PSP, CBS AD < bvFTD, PD

A) No group differences between cohort for demographics, MMSE and CDRsb. B) Group differences for the combined cohorts for demographics and neuropsychological assessments. Each cell shows mean (standard deviation); Group comparison with post-hoc analysis, significant at $p < 0.05$. Abbreviations= ns: not significant; see text for other abbreviations.