

Neurofilament relates to white matter microstructure in older adults

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Abstract

Cerebrospinal fluid (CSF) neurofilament light (NFL) is a **protein** biomarker hypothesized to reflect axonal injury. To study whether NFL is associated with diffusion tensor imaging (DTI) measurements of white matter (WM) microstructure, Vanderbilt Memory & Aging Project participants with normal cognition (NC, n=77), early mild cognitive impairment (**eMCI**, n=15), and MCI (n=55) underwent lumbar puncture to obtain CSF and multimodal 3T brain MRI. Voxel-wise analyses cross-sectionally related NFL to DTI metrics, adjusting for demographic and vascular risk factors. Increased NFL correlated with **multiple DTI metrics** (p-values<.048). There was an NFL x diagnosis interaction (**excluding eMCI**) on WM microstructure (p-values<.05), with associations strongest among MCI. Post-hoc models revealed NFL x CSF amyloid- β_{42} and NFL x CSF tau interactions relative to WM measures (p-values<.05), with associations strongest among amyloid negative and tau positive. Findings suggest increased NFL, a biomarker of axonal injury, is correlated with compromised WM microstructure. **Results highlight the role of elevated NFL in predicting WM damage in cognitively impaired older adults who are amyloid negative and tau positive.**

Key Words: Neurofilament Light, Diffusion Tensor Imaging, Cerebrospinal Fluid, Alzheimer's Disease

Abbreviations:

NFL	Neurofilament light	FA	Fractional Anisotropy
MCI	Mild cognitive impairment	FMRIB	Functional magnetic resonance imaging of the brain
DTI	Diffusion tensor imaging	FSL	FMRIB software library
NC	Normal cognition	FSRP	Framingham Stroke Risk Profile
eMCI	Early mild cognitive impairment	CVD	Cardiovascular disease
Aβ_{42}	Amyloid- β_{42}	APOE	Apolipoprotein E
T-tau	Total tau	SNAP	Suspected non-amyloid pathology
TBSS	Tract based spatial statistics		

1. Introduction

One rapidly emerging biomarker in abnormal cognitive aging and Alzheimer's disease is NFL, a support protein within **large, myelinated** axons specifically within the cerebral white matter (Bergman et al., 2016; Friede and Samorajski, 1970; Skillback et al., 2014; Yates et al., 2009). Increased CSF NFL concentrations are thought to reflect breakdown of large caliber axons (Friede and Samorajski, 1970; Menke et al., 2015), thus compromising white matter microstructure with cognitive consequences (Menke et al., 2015; Skillback et al., 2014). Elevated CSF NFL correlates with MRI T1-weighted white matter hypointensities, reflecting white matter macrostructural damage, particularly among older adults with MCI and Alzheimer's disease (Zetterberg et al., 2016). **Prior studies have yielded limited and inconsistent associations between CSF NFL and white matter microstructure assessed on DTI among middle aged and older individuals with normal cognition (Melah et al., 2016; Racine et al., 2017). Therefore, it is unknown if CSF NFL relates to more sensitive measures of white matter specific to the microstructure of fiber bundles, among older adults with and without cognitive impairment.**

The current study sought to examine the association between CSF NFL obtained *in vivo* and DTI measures of white matter microstructure among older individuals with NC and MCI, a prodromal stage of Alzheimer's disease. Given prior research linking CSF NFL, axonal injury, and white matter integrity in murine models (Friede and Samorajski, 1970) and motor neuron disease (Menke et al., 2015), we hypothesized that increased CSF NFL concentrations would correlate with compromised white matter microstructure. We also hypothesized effect modification by cognitive diagnosis with associations stronger in MCI, where compromised axonal integrity may underlie clinical signs of cognitive impairment (Nir et al., 2013) **and a greater range of white matter degeneration is present (Huang et al., 2007).** In post-hoc analyses,

NFL and white matter microstructure associations were tested for interactions with established Alzheimer's disease biomarkers, including CSF A β ₄₂ and t-tau to determine if underlying Alzheimer's disease pathology was driving any significant observations.

2. Materials and Methods

2.1 Study Cohort

The Vanderbilt Memory & Aging Project (Jefferson et al., 2016) is a longitudinal observational study investigating vascular health and brain aging, enriched with older adults with MCI (Albert et al., 2011). Cohort inclusion criteria required participants be at least 60 years of age, speak English, have adequate auditory and visual acuity for testing, and have a reliable study partner. At eligibility, participants underwent a comprehensive assessment and were excluded for a cognitive diagnosis other than NC, **eMCI** (Aisen et al., 2010), or MCI (Albert et al., 2011), MRI contraindication, history of neurological disease (e.g., stroke), heart failure, major psychiatric illness, head injury with loss of consciousness >5 minutes, or a systemic or terminal illness affecting follow-up participation. At enrollment, participants completed a comprehensive examination including (but not limited to) fasting blood draw, physical examination, clinical interview, echocardiogram, multi-modal brain MRI, and optional lumbar puncture. Participants were excluded from the current study for missing CSF NFL, missing DTI, or missing covariate data (**Fig. 1**). The protocol was approved by the Vanderbilt University Medical Center Institutional Review Board. Written informed consent was obtained prior to data collection.

2.2 Lumbar Puncture & Biochemical Analyses

A subset of participants completed an optional fasting lumbar puncture at enrollment (n=155, **Fig. 1**). CSF was collected with polypropylene syringes using a Sprotte 25-gauge spinal needle in an intervertebral lumbar space. Samples were immediately mixed and centrifuged. Supernatants were aliquoted in 0.5 mL polypropylene tubes and stored at -80°C. Samples were

analyzed in **single** batch using a commercially available enzyme-linked immunosorbent assay (Uman Diagnostics) to measure CSF NFL concentration. Commercially available enzyme-linked immunosorbent assays (Fujirebio, Ghent, Belgium) were used to measure CSF concentrations of $A\beta_{42}$ (INNOTEST[®] β -AMYLOID₍₁₋₄₂₎) and t-tau (INNOTEST[®] hTAU). Board certified laboratory technicians processed data blinded to clinical information (Palmqvist et al., 2014). Intra-assay coefficients of variation were <10%.

2.3 Brain MRI Acquisition & Post-Processing

Participants were scanned at the Vanderbilt University Institute of Imaging Science on a 3T Philips Achieva system (Best, The Netherlands) using an 8-channel SENSE reception coil array as part of a multi-modal acquisition protocol. DTI data were acquired along 32 diffusion gradient vectors (TR/TE=10000/60ms, spatial resolution=2x2x2mm³, b-value=1000s/mm²) **with one non-diffusion weighted image (B₀)** and post-processed through an established tract-based spatial statistics (TBSS) pipeline using the FSL version 4.1.4 (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSL>) (Smith et al., 2006).

Data were corrected for motion and eddy currents, a brain mask was created, the diffusion tensor model was fit using FMRIB's Diffusion Toolbox, and FA, mean diffusivity, radial diffusivity, and axial diffusivity values were calculated. All FA images were non-linearly registered, merged into a 4D image, and a mean image was created. The mean image was used to generate a mean skeleton, to which a threshold was applied to exclude voxels that did not overlap among 80% or more of participants. Each participant's FA image was projected onto the mean skeleton, and these skeleton projections were combined into one 4D file containing all skeletonized FA data from all participants. Nonlinear registration was also applied to the mean

diffusivity, radial diffusivity, and axial diffusivity images for each participant. For each individual metric, participant data were merged into one 4D file that was projected onto the original mean FA skeleton.

2.4 Analytical Plan

Covariate information was documented at enrollment. Systolic blood pressure was the mean of two measurements. Diabetes mellitus was defined as current fasting blood glucose ≥ 126 mg/dL, hemoglobin A1C $\geq 6.5\%$, or current oral hypoglycemic or insulin medication usage. Medication review determined anti-hypertensive medication use. Left ventricular hypertrophy was defined on echocardiogram (left ventricle mass index > 115 g/m² in men, > 95 g/m² in women). Self-report atrial fibrillation was corroborated by any of the following sources: echocardiogram, documented prior procedure/ablation for atrial fibrillation, or medication usage for atrial fibrillation. Current cigarette smoking (yes/no within the year prior to enrollment examination) was ascertained by self-report. Self-report prevalent CVD with supporting medical record evidence included coronary heart disease, angina, or myocardial infarction (note, heart failure was a parent study exclusion). FSRP score was calculated by applying points by sex for age, systolic blood pressure, anti-hypertensive medication usage, diabetes mellitus, current cigarette smoking, left ventricular hypertrophy, atrial fibrillation, and CVD (D'Agostino et al., 1994). *APOE* genotyping was quantified from DNA extracted from whole blood samples (Jefferson et al., 2016). *APOE-ε4* carrier (*APOE4*) status was defined as positive ($\epsilon 2/\epsilon 4$, $\epsilon 3/\epsilon 4$, $\epsilon 4/\epsilon 4$) or negative ($\epsilon 2/\epsilon 2$, $\epsilon 2/\epsilon 3$, $\epsilon 3/\epsilon 3$).

Prior to analyses, CSF NFL data were visually inspected to ensure data were normally distributed and participants with outlying NFL values (≥ 5 standard deviations away from the

sample mean) were excluded. Voxel-wise analyses were conducted using the FSL randomise procedure with 5000 permutations. General linear models using permutation testing related CSF NFL concentrations (pg/mL) to FA, mean diffusivity, radial diffusivity, and axial diffusivity, adjusting for age, education, race/ethnicity, FSRP (excluding points assigned to age), cognitive diagnosis, and *APOE4* status. Models were repeated evaluating a CSF NFL x cognitive diagnosis interaction on DTI metrics. Excluding participants with eMCI, models were repeated stratifying by cognitive diagnosis (NC, MCI).

In post-hoc analyses, unadjusted Pearson correlations were generated between NFL, $A\beta_{42}$, and t-tau, and formal collinearity analysis was performed by calculating a variation inflation index. In fully adjusted models including all participants, general linear models related CSF $A\beta_{42}$ and t-tau concentrations to FA, mean diffusivity, radial diffusivity, and axial diffusivity. Additionally, CSF NFL x $A\beta_{42}$ (amyloid), CSF NFL x t-tau (neurodegeneration), and CSF NFL x *APOE4* status interaction terms were individually evaluated on FA, mean diffusivity, radial diffusivity, and axial diffusivity. Models were repeated stratifying by amyloid positivity ($A\beta_{42}\leq 530$ pg/mL, n=42) versus negativity ($A\beta_{42}>530$ pg/mL, n=105) (Hansson et al., 2006) and by t-tau positivity (t-tau ≥ 400 pg/mL, n=65) versus negativity (t-tau <400 pg/mL, n=82) (Baldacci et al., 2017). Using existing definitions (Jack et al., 2012; Sperling et al., 2011), all participants were classified into four groups based on a combination of amyloid and t-tau status, including amyloid and neurodegeneration positive ($A\beta_{42+}/t\text{-tau+}$, n=31), amyloid positive only ($A\beta_{42+}/t\text{-tau-}$, n=11), t-tau positive only (commonly referred to as *Suspected Non-Amyloid Pathology* or SNAP; $A\beta_{42-}/t\text{-tau+}$, n=34), or amyloid and neurodegeneration negative ($A\beta_{42-}/t\text{-tau-}$, n=71). Models were repeated stratifying by these four biomarker status groups. Finally, within the MCI group only, models were repeated stratified by amyloid positivity (n=25) versus negativity

(n=30), t-tau positivity (n=32) versus negativity (n=23), and stratified by the four combined biomarker groups ($A\beta_{42+}/t\text{-tau+}$, n=20; $A\beta_{42+}/t\text{-tau-}$, n=5; $A\beta_{42-}/t\text{-tau+}$, n=12; $A\beta_{42-}/t\text{-tau-}$, n=18).

Correction for multiple comparisons was performed using the established cluster enhancement permutation procedure in FSL (Smith and Nichols, 2009), and the threshold for statistical significance was set *a priori* as corrected p-value<.05. Sensitivity analyses and parametric estimates of statistically significant associations were calculated in R version 3.2.1 (www.r-project.org) using least squares regression for illustration and interpretation purposes.

3. Results

3.1 Participant Characteristics

For all participants (n=147, 72±6 years, 68% male, 93% non-Hispanic White), CSF NFL concentrations ranged 268 to 3617 pg/mL, CSF A β ₄₂ concentrations ranged 289 to 1195 pg/mL, and CSF t-tau concentrations ranged 107 to 1542 pg/mL. NFL was not correlated with A β ₄₂ (r=-0.05, p=.55) and was correlated with t-tau (r=0.42, p<.001). **No collinearity was found between the CSF variables (Variation Inflation Index <2.9 for all variables in all models).** Mean time between brain MRI and lumbar puncture was 37±41 days. See **Table 1** for participant characteristics for the entire sample and stratified by diagnosis. See **Supplementary Table 1** for participant characteristics stratified by amyloid and t-tau status.

3.2 CSF Biomarkers & DTI Metrics

Among all participants, NFL was negatively correlated with FA primarily in the striatum (corrected p-values<.048). Similarly, NFL was positively associated with mean diffusivity in the **white matter underlying the** fusiform gyrus (corrected p-value=.002), radial diffusivity in the striatum (corrected p-value=.002), and axial diffusivity in **the white matter underlying the** straight gyrus (corrected p-value=.002) (**Fig. 2, Table 2, Supplementary Figure 1**). **A β ₄₂ was associated with mean diffusivity and axial diffusivity (corrected p-values< .001, Supplementary Table 2).** T-tau was not associated with DTI metrics (corrected p-values>.18).

NFL interacted with cognitive diagnosis on FA in the superior corona radiata (corrected p-value=.01), mean diffusivity in the posterior thalamic radiation (corrected p-value=.003), radial diffusivity in **the white matter underlying** the precentral gyrus (corrected p-values<.05), and axial diffusivity in the inferior fronto-occipital fasciculus (corrected p-value=.002)

(**Supplementary Table 3**). Diagnostic stratification revealed NFL was only **positively** associated with radial diffusivity in the posterior thalamic radiation (corrected p-value=.048) among NC participants. In contrast, among MCI participants, NFL was associated with all DTI metrics, including negative associations with FA in the superior corona radiata and posterior thalamic radiation (corrected p-values<.05) and positive associations with mean diffusivity in the anterior corona radiata (corrected p-values<.049), radial diffusivity in the striatum (corrected p-values<.02), and axial diffusivity in the anterior corona radiata (corrected p-value=.011) (**Fig. 3, Supplementary Table 4**).

3.3 NFL x Alzheimer's Disease Biomarker Interactions & DTI Metrics

All NFL x Alzheimer's disease CSF biomarker interaction results are presented in **Supplementary Table 5**. NFL interacted with A β ₄₂ on FA (corrected p-values<.05) and radial diffusivity (corrected p-values<.05), but not mean or axial diffusivity (corrected p-values>.062). Interactions reflected a stronger association between **elevated CSF NFL** and **worse** DTI metrics among individuals with higher CSF A β ₄₂ levels (**Fig. 3, Supplementary Table 6, Supplementary Figure 2**).

NFL interacted with t-tau on FA (corrected p-value=.034), mean diffusivity (corrected p-values<.046), radial diffusivity (corrected p-values<.05), and axial diffusivity (corrected p-values<.047). Interactions reflected a stronger association **between elevated CSF NFL** and **worse** DTI metrics among individuals with higher t-tau levels (**Fig. 3, Supplementary Table 7, Supplementary Figure 3**). NFL did not interact with *APOE4* status on DTI metrics (corrected p-values>.07).

3.4 NFL & DTI Metrics by Alzheimer's Disease Biomarker Status

Given the strongest NFL associations were present among amyloid negative but t-tau positive participants, post-hoc analyses examined NFL associations among biomarker groups defined using preclinical Alzheimer's disease criteria (Jack et al., 2012; Sperling et al., 2011). NFL was not associated with any DTI metric among amyloid and neurodegeneration positive participants ($A\beta_{42+}/t\text{-tau+}$, corrected p-values $>.27$). NFL was only associated with axial diffusivity among amyloid positive only participants ($A\beta_{42+}/t\text{-tau-}$, corrected p-value = .037). Among t-tau positive only participants ($A\beta_{42-}/t\text{-tau+}$ or SNAP), NFL was negatively associated with FA (corrected p-values $<.05$) and positively associated with mean diffusivity (corrected p-value = .005), radial diffusivity (corrected p-value = .002), and axial diffusivity (corrected p-value = .035). Among amyloid and neurodegeneration negative participants ($A\beta_{42-}/t\text{-tau-}$), NFL was positively associated with mean diffusivity (corrected p-values $<.05$), radial diffusivity (corrected p-values $<.048$), and axial diffusivity (corrected p-values $<.05$). NFL was not associated with FA (corrected p-values >0.11 , **Supplementary Table 8**).

3.5 NFL & DTI metrics by Alzheimer's Disease Biomarker Status Among MCI

Among MCI participants, NFL was not associated with any DTI metric among amyloid positive participants (corrected p-values $>.08$). NFL was associated with DTI metrics among amyloid negative (corrected p-values $<.05$), t-tau positive (corrected p-values $<.049$), and t-tau negative participants (corrected p-values $<.05$). NFL was not associated with DTI metrics among amyloid and neurodegeneration positive ($A\beta_{42+}/t\text{-tau+}$), amyloid positive only ($A\beta_{42+}/t\text{-tau-}$), or t-tau positive only participants ($A\beta_{42-}/t\text{-tau+}$, corrected p-values $>.06$). NFL was associated with DTI metrics among amyloid and neurodegeneration negative participants ($A\beta_{42-}/t\text{-tau-}$,

corrected p-values<.049). All results are presented in **Supplementary Table 9**.

4. Discussion

Among community-dwelling older adults without a clinical history of stroke or dementia, higher CSF concentrations of NFL were associated with compromised white matter microstructure measured with DTI. Specifically, NFL was negatively associated with FA and positively associated with mean, radial, and axial diffusivity. Diagnostic interactions revealed findings were strongest in the MCI participants. Biomarker interactions including both NC and MCI participants further indicated that findings were strongest among amyloid negative and t-tau positive individuals. Collectively, these results suggest that the association between higher CSF concentrations of NFL, a biomarker of axonal degradation, and white matter microstructure is modified by cognitive diagnosis and CSF biomarker profile.

The current findings contribute novel information to an emerging literature on CSF and neuroimaging markers of white matter microstructure in individuals at risk for clinical dementia (Bendlin et al., 2012; Melah et al., 2016). Among the entire cohort, associations were primarily detected in the striatum, fusiform gyrus, and straight gyrus, an inferior section of the frontal lobe. This distribution extending to most of the white matter and present among all four DTI metrics likely represents global white matter damage. NFL is a protein that contributes to the structure of neurofilaments, which are responsible for supporting axonal integrity and neuronal transport (Friede and Samorajski, 1970; Yates et al., 2009). Elevated CSF NFL concentrations may reflect structural or metabolic changes in axons due to a variety of causes, such as cerebral inflammation (Melah et al., 2016), subclinical neural trauma (Zetterberg et al., 2006), and neurodegenerative disease (Skillback et al., 2014). As mechanical damage disrupts the integrity of the axonal membrane, neurofilaments enter the CSF, resulting in increased CSF NFL levels (Petzold, 2005). In addition, chemical changes, such as calcium dysregulation or increased intra-

axonal calcium concentration, may lead to neurofilament disruption and compaction. These chemical changes may promote the movement of neurofilaments into the CSF when mechanical damage is present (Marmarou and Povlishock, 2006). Thus, CSF NFL concentrations likely indicate global axonal damage that is also reflected in imaging measures sensitive to white matter microstructure.

Although NFL reflects axonal damage and is possibly more specific to changes in axial diffusivity (Song et al., 2002), NFL was associated with changes in all four DTI metrics. FA and mean diffusivity are considered sensitive markers of white matter microstructure, though not necessarily specific to any single mechanism of damage (Sen and Basser, 2005). Radial diffusivity is hypothesized to primarily reflect changes in myelination (Song et al., 2002), which affect axonal transport (Sun et al., 2016). Therefore, axonal damage indicated by an increase in NFL may be due to a variety of etiologies, including demyelination, and could be expected to relate to all four DTI metrics. Additionally, as seen in the skeleton images (Fig 2, Panel B), the associations in the whole sample represent a global effect and are not specific to one region. In the MCI group, associations were most prominent in the corona radiata, specifically in tracts within the temporal lobe, where Alzheimer's disease pathology develops (Scholl et al., 2016). Associations in the SNAP group were strongest in tracts within the temporal lobe, suggesting that these associations may reflect regions susceptible to tauopathy in the absence of amyloidosis.

While NFL only related to radial diffusivity in one small white matter region in the NC group, among the MCI group, higher levels of NFL were associated with compromise in all white matter microstructure metrics across a majority of white matter tracts. The strongest associations in the MCI cohort occurred in the superior corona radiata, striatum, and anterior

corona radiata, regions that are susceptible to damage in MCI (Ren et al., 2016; Thillainadesan et al., 2012). This observation suggests that a higher threshold of axonal damage, as seen in MCI (Huang et al., 2007), may be necessary before CSF NFL corresponds to white matter microstructure captured on neuroimaging. **Additionally, this association may have clinical utility in determining the etiology of MCI. Elevated CSF NFL in those with MCI may indicate white matter damage as a potential etiology of the cognitive impairment, rather than other pathology.** Early and more precise detection of neuropathology underlying cognitive impairment would facilitate the delivery of therapies targeted toward specific etiologies.

Our results also demonstrate that CSF biomarkers of Alzheimer's disease neuropathology may modify the association between NFL and DTI. The NFL x $A\beta_{42}$ interaction on white matter microstructure was driven by a strong association between NFL and DTI metrics among the amyloid negative group, whereas results were null in the amyloid positive group. **Additionally, stratified results among those participants with MCI were null in the amyloid positive group.** The NFL x t-tau interaction on DTI was driven by a strong association in the tau positive group with associations strongest in participants meeting biomarker criteria for SNAP. Prior work in a group of middle aged and older adults with NC (many of whom had a positive family history of Alzheimer's disease) showed an interaction between CSF NFL and phosphorylated tau for one small white matter microstructural area (Melah et al., 2016). Findings presented here extend this prior preliminary result by showing a robust interaction between t-tau and white matter microstructure among NC and symptomatic (MCI) older adults. Plus, stratified analyses suggest elevated CSF NFL may reflect wide-spread white matter damage among tau positive individuals or those individuals with SNAP. Taken together, results across all four DTI metrics presented here suggest that elevated CSF NFL may represent axonopathy due to a non-amyloid pathway of

injury, particularly among individuals with MCI, elevated CSF tau, or SNAP. **One possibility is that individuals with MCI who are amyloid negative may be more likely to have different pathological processes, contributing to cognitive impairment and white matter damage.** In the absence of amyloid, this scenario is consistent with recent work suggesting that adverse vascular (Hohman et al., 2015) and endocrine health (Lane et al., 2016) have stronger effects on brain integrity in the absence of enhanced Alzheimer's disease biomarkers.

The current study has several strengths, including a clinically well characterized cohort emphasizing participants with normal cognition and prodromal dementia and excellent methods for quantifying white matter microstructure and CSF biomarkers of AD, neurodegeneration, and axonal injury. Additional strengths include comprehensive ascertainment of potential confounders and the application of a cluster enhancement permutation procedure to correct for multiple comparisons, thereby reducing the possibility of a false positive finding. Finally, core laboratories using quality control procedures analyzed all MRI and CSF measurements in batch, and technicians were blinded to participant clinical information. Despite these strengths, the study is cross-sectional and cannot address causality. Longitudinal studies are needed to understand the temporal nature of associations reported here. **Due to the lack of concordance between SNAP classifications using hippocampal volume on MRI versus CSF tau and our emphasis on how CSF NFL interacts with amyloid and tau, SNAP analyses presented here relied on a CSF tau definition. Neurodegeneration defined by changes in hippocampal volume can be attributed to a variety of pathophysiologies, such as hippocampal sclerosis (Jackson et al., 1990) and TAR DNA-binding protein 43 (Josephs et al., 2017). Though results may not be generalized to neuroimaging definitions of SNAP, the definition used here allows conclusions to be drawn that are specific to tau pathology.** Also, the cohort was predominantly non-Hispanic White with

participants 60 to 92 years of age, thus limiting generalizability to other races, ethnicities, and age groups.

5. Conclusions

The current study demonstrates a novel association between NFL and white matter microstructure, which is modified by cognitive diagnosis (MCI) and biomarkers of Alzheimer's disease ($A\beta_{42}$) and neurodegeneration (t-tau). Many older adults have comorbid pathologies present in late life (Schneider et al., 2007) and potential Alzheimer's disease therapies may be less effective when additional pathologies are present (Weekman et al., 2016). Therefore, the modified associations reported here between NFL and DTI in the presence or absence of AD pathology may have clinical significance for more targeted therapies as effective interventions emerge. Additional research is needed to delineate the underlying mechanism(s) of associations reported here, examine biomarker associations in a larger cohort stratified by diagnostic group, and better understand what neuropathological processes underlie cognitive impairment observed in amyloid negative individuals. Future analyses should also incorporate plasma NFL, an emerging blood biomarker that correlates with CSF NFL (Mattsson et al., 2017).

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Declarations of Interest

None

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Figure Legends

Figure 1. Participant Inclusion/Exclusion Details

Figure 1. Missing data categories are mutually exclusive. Of the 6 participants excluded for missing NFL data, 1 was defined as an outlier. CSF=cerebrospinal fluid; NFL=neurofilament light; DTI=diffusion tensor imaging; NC=normal cognition; MCI=mild cognitive impairment; T-tau=total tau.

Figure 2. NFL & DTI Results

Figure 2. Panel A: Association between NFL and fractional anisotropy. Mean FA skeleton shows regions where NFL is associated with FA. Scatterplot shows least squares regression relating mean FA values for every participant at one specific cluster and NFL levels. **Panel B:** Association between NFL and diffusivity metrics. Mean FA skeletons show regions where NFL is associated with mean, radial, and axial diffusivity. Parametric p-values, β , and region listed only represent the cluster with the lowest p-value for each DTI metric. All skeleton images taken at Z=91. NFL=neurofilament light; DTI=diffusion tensor imaging; FA=fractional anisotropy.

Figure 3. NFL & Fractional Anisotropy by Diagnosis, Amyloid Status, & T-Tau Status

Figure 3. Mean FA skeletons show regions where NFL is associated with FA in models stratified by cognitive diagnosis, amyloid status, and t-tau status. Significance is seen in MCI, amyloid negative, and t-tau positive only. All skeleton images taken at Z=91. NFL=neurofilament light; DTI=diffusion tensor imaging; NC=normal cognition; MCI=mild cognitive impairment; T-tau=total tau.

Table 1. Participant Demographic, Biomarker, & DTI Characteristics

	Total n=147	NC n=77	eMCI n=15	MCI n=55	p-value
Demographic & Health Characteristics					
Age (years)	72±6	72±7	73±6	73±6	0.67
Sex (% male)	68	71	80	60	0.22
Race (% Non-Hispanic White)	92	94	93	91	0.85
Education (years)	16±3	17±2	16±3	15±3	0.001*
<i>APOE4</i> (% positive)	33	29	13	45	0.03‡
FSRP, total score ^a	12±4	11±4	13±3	12±4	0.16
Systolic blood pressure, mmHg	142±16	139±15	148±15	146±17	0.03†*
Antihypertensive medication usage (%)	46	48	40	46	0.84
Diabetes (%)	18	13	27	22	0.27
Current smoking (%)	1	0	7	2	0.12
Left ventricular hypertrophy (%)	3	1	7	5	0.33
Atrial fibrillation (%)	3	5	0	0	0.15
Prevalent CVD (%)	3	4	0	2	0.61
Time between MRI and CSF Acquisition (days)	37±41	35±43	31±23	41±44	0.58
CSF Biomarkers, pg/mL					
Neurofilament Light	1048±529	931±451	1088±465	1200±609	0.01*
Aβ ₄₂	717±244	767±225	817±282	619±231	<0.001‡*
T-tau	432±229	379±175	429±125	506±292	0.006*

Note. Values denoted as mean±standard deviation or frequency. **P-values were generated using a one-way ANOVA.** †NC is different than eMCI. ‡eMCI is different than MCI. *NC is different than MCI. ^aa modified FSRP score was included in statistical models excluding points assigned to age (5.7±2.1). *APOE*=apolipoprotein E; CSF=cerebrospinal fluid; FSRP=Framingham Stroke Risk Profile; CVD=cardiovascular disease.

Table 2. Region Specific NFL Associations with DTI Metrics

	Anatomical Region	Hemisphere	Volume (mm ³)	Cluster Statistics		Corrected p-value ^c	MNI Coordinate ^d		
				β^a	p-value ^b				
Fractional Anisotropy	Striatum	Right	11857	-0.438	4.16x10 ⁻⁸	0.007	41	-39	-9
	Superior Longitudinal Fasciculus	Left	8213	-0.437	7.95x10 ⁻⁸	0.009	-26	-4	27
	Posterior Limb of the Internal Capsule	Left	125	-0.306	6.56x10 ⁻⁴	0.048	-20	-9	6
Mean Diffusivity	Fusiform Gyrus	Right	59442	0.388	8.92x10 ⁻⁷	0.002	38	-38	-18
Radial Diffusivity	Striatum	Right	58919	0.377	1.39x10 ⁻⁶	0.002	40	-33	-14
Axial Diffusivity	Straight Gyrus	Right	44448	0.508	3.79x10 ⁻¹⁰	0.002	8	47	-20

Note. Gyri refer to the white matter underlying that region. ^a β is standardized; ^bparametric p-values were calculated using least squares regression to relate raw DTI values extracted from each participant skeleton and NFL; ^cp-values have been corrected for multiple comparisons; ^dcoordinates represent the voxel with the minimum p-value in each cluster; NFL=neurofilament light; DTI=diffusion tensor imaging.