

Stage-specific links between plasma neurofilament light and imaging biomarkers of Alzheimer's disease

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Neurofilament light (NfL) is a marker of neuroaxonal injury, a prominent feature of Alzheimer's disease. It remains uncertain, however, how it relates to amyloid and tau pathology or neurodegeneration across the Alzheimer's disease continuum. The aim of this study was to investigate how plasma NfL relates to amyloid and tau PET and MRI measures of brain atrophy in participants with and without cognitive impairment. We retrospectively examined the association between plasma NfL and MRI measures of grey/white matter volumes in the Alzheimer's Disease Neuroimaging Initiative [ADNI: $n = 1149$; 382 cognitively unimpaired control subjects and 767 cognitively impaired participants (mild cognitive impairment $n = 420$, Alzheimer's disease dementia $n = 347$)]. Longitudinal plasma NfL was measured using single molecule array (Simoa) technology. Cross-sectional associations between plasma NfL and PET amyloid and tau measures were independently assessed in two cohorts: ADNI [$n = 198$; 110 cognitively unimpaired, 88 cognitively impaired (MCI $n = 67$, Alzheimer's disease dementia $n = 21$), data accessed October 2018]; and Translational Biomarkers in Aging and Dementia [TRIAD, $n = 116$; 74 cognitively unimpaired, 42 cognitively impaired (MCI $n = 16$, Alzheimer's disease dementia $n = 26$), data obtained November 2017 to January 2019]. Associations between plasma NfL and imaging-derived measures were examined voxel-wise using linear regression (cross-sectional) and linear mixed effect models (longitudinal). Cross-sectional analyses in both cohorts showed that plasma NfL was associated with PET findings in brain regions typically affected by Alzheimer's disease; associations were specific to amyloid PET in cognitively unimpaired and tau PET in cognitively impaired ($P < 0.05$). Longitudinal analyses showed that NfL levels were associated with grey/white matter volume loss; grey matter atrophy in cognitively unimpaired was specific to *APOE* $\epsilon 4$ carriers ($P < 0.05$). These findings suggest that plasma NfL increases in response to amyloid-related neuronal injury in preclinical stages of Alzheimer's disease, but is related to tau-mediated neurodegeneration in symptomatic patients. As such, plasma NfL may a useful measure to monitor effects in disease-modifying drug trials.

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Abbreviations: LME = linear mixed effect; MCI = mild cognitive impairment; NfL = neurofilament light

Introduction

Alzheimer's disease is biologically characterized by the accumulation of extracellular amyloid- β plaques and intracellular tau aggregates. These pathological hallmarks are thought to result in neurodegenerative changes via direct and synergistic effects, leading, ultimately, to cognitive impairment and functional decline. Increasingly, biomarkers of these biological processes are used in both clinical practice and research settings and in the context of clinical trials and drug development. The most commonly used Alzheimer's disease biomarkers include structural MRI-based measures of atrophy, PET-based imaging of brain glucose metabolism, amyloid- β , and tau tangles, and CSF measures of amyloid- β , tau and neuronal injury. Hampering the use of these biomarkers, however, is the high cost and limited availability of imaging-based measures, especially PET, and the perceived invasiveness of CSF sampling. As a result, blood-based biomarkers are increasingly seen as a simplified initial screening step in primary care (Hampel *et al.*, 2018). One such potential measure is the axonal injury marker neurofilament light (NfL) (Zetterberg, 2016). NfL is a key structural component of the neuronal cytoskeleton and is abundantly expressed in large-calibre myelinated axons (Trojanowski *et al.*, 1986). In response to injury, NfL is released into the CSF and blood; biofluid concentrations of NfL, however, also increase in an age-dependent manner (Bridel *et al.*, 2019). NfL has been shown to be elevated in both plasma (Mattsson *et al.*, 2019) and CSF (Sjogren *et al.*, 2001) in sporadic, familial and preclinical Alzheimer's disease (Weston *et al.*, 2017; Preische *et al.*, 2019; Quiroz *et al.*, 2020), and to correlate with cognitive, biochemical, imaging-based measures and

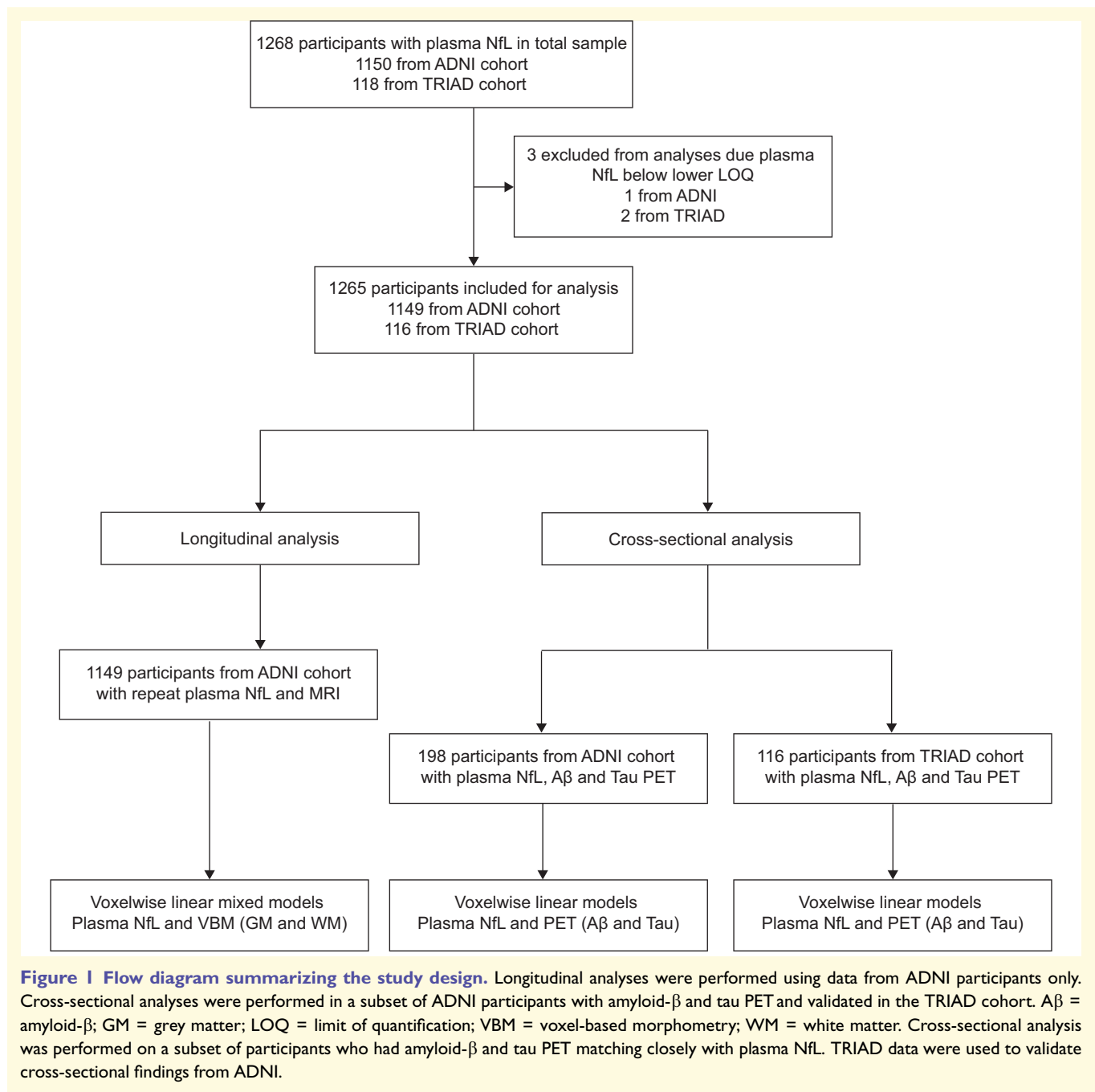
post-mortem findings in Alzheimer's disease (Zetterberg *et al.*, 2016; Mattsson *et al.*, 2017, 2019; Ashton *et al.*, 2019). However, studies addressing associations between plasma NfL and imaging markers are few (Zetterberg *et al.*, 2016; Mattsson *et al.*, 2017, 2019; Chatterjee *et al.*, 2018; Ashton *et al.*, 2019; Benedet *et al.*, 2019; Thijssen *et al.*, 2020), and these have mostly been confined to explore pre-defined regions of interest and a global index of white matter change.

With consideration for the above, here we investigated the association between plasma NfL concentrations and cross-sectional amyloid- β and tau PET as well as longitudinal MRI-derived measures of grey matter and white matter atrophy in a large number of cognitively unimpaired and cognitively impaired individuals [mild cognitive impairment (MCI) and dementia due to Alzheimer's disease]. As plasma NfL alterations are not considered specific to a particular disease but are instead linked to neurodegeneration processes, we hypothesized that, across the clinical spectrum of Alzheimer's disease, plasma NfL would be differentially related to amyloid- β and tau pathology. We also hypothesized that plasma NfL would be associated with progressive grey and white matter atrophy.

Materials and methods

Study design and participants

This retrospective study was based on data from the ADNI (Alzheimer's disease Neuroimaging Initiative; data downloaded October, 2018) and TRIAD (Translational Biomarkers in Aging



and Dementia; data collected between November, 2017 and January, 2019) cohorts as described in Fig. 1 and the Supplementary material. In ADNI, cognitively unimpaired participants had a Mini-Mental State Examination (MMSE) score ≥ 24 and a Clinical Dementia Rating (CDR) score of 0. The cognitively impaired were defined as MCI if they had objective memory loss based on delayed recall performance on the Wechsler Memory Scale (logical memory II) [>1 standard deviation (SD) below the mean], MMSE scores ≥ 24 , a CDR score of 0.5 and preserved activities of daily living (i.e. no dementia). Cognitively impaired individuals with Alzheimer's disease dementia fulfilled the National Institute of Neurological Communicative Disorders and Stroke–Alzheimer Disease and

Related Disorders Association (NINCDS-ADRDA) criteria for probable Alzheimer's disease (McKhann *et al.*, 1984), had MMSE scores between 20 and 26, and CDR scores between 0.5 and 1. In TRIAD, cognitively unimpaired and cognitively impaired groups were defined using the same criteria as in ADNI, though the Alzheimer's disease participants had a CDR between 1 and 2. Exclusion criteria for both cohorts included the presence of medical contraindications and being enrolled concurrently in other trials or studies.

Longitudinal analysis was performed using ADNI data only; cross-sectional analyses were performed in both cohorts, with the TRIAD cohort included to validate ADNI-based findings. The same inclusion/exclusion criteria were used to define

cognitively unimpaired and cognitively impaired groups in ADNI and TRIAD. For the longitudinal analysis in ADNI, longitudinal plasma NfL (up to 48 months) and MRI-based measures (closest in time to plasma collection) were obtained for 1149 participants [including 382 cognitively unimpaired controls and 767 cognitively impaired participants (MCI, $n = 420$; Alzheimer's disease dementia, $n = 347$)], as described below. The cross-sectional (ADNI-based) analysis between plasma NfL and PET-based measures of amyloid- β (^{18}F -florbetapir) and tau (^{18}F -flortaucipir), was also investigated using a subset of participants [$n = 198$, including 110 cognitively unimpaired controls and 88 cognitively impaired participants (MCI $n = 67$; Alzheimer's disease dementia $n = 21$)]. This cross-sectional analysis was subsequently replicated in 116 participants from the TRIAD cohort [including 74 cognitively unimpaired controls and 42 cognitively impaired participants (MCI $n = 16$; Alzheimer's disease dementia $n = 26$)] with plasma NfL, amyloid- β (^{18}F -AZD4694) and tau (^{18}F -MK6240) PET. For these ADNI and TRIAD-based cross-sectional analyses, selected PET scans were those closest in time to plasma NfL collection.

Regional ethical committees of all participating institutions approved the ADNI study. The Research Ethics Board of the Montreal Neurological Institute as well as the Faculty of Medicine Research Ethics Office, McGill University, approved the TRIAD study. All study participants provided written informed consent.

Plasma quantification

For both cohorts, plasma NfL concentrations were measured using an in-house immunoassay on the Single molecule array (Simoa) HD-1 Analyser (Quanterix), as previously described (Gisslen *et al.*, 2016). Measurements were performed using a single batch of reagents for each cohort (Supplementary material).

Voxel-based morphometry

For ADNI, 1.5T and 3T T_1 -weighted MRI preprocessed scans were segmented into probabilistic grey and white matter maps. These were then non-linearly registered to the ADNI template and smoothed with a Gaussian kernel. For further details pertaining to image preprocessing, see the Supplementary material.

Brain amyloid- β and tau imaging

In the ADNI cohort, amyloid- β load was estimated using ^{18}F -florbetapir (40–70 min post-injection) standardized uptake value ratio (SUVR) and the cerebellar grey matter as reference region.

Tau load was estimated using ^{18}F -flortaucipir (80–100 min post-injection) SUVR, using the inferior cerebellar grey matter as reference region. Full PET details are described in the Supplementary material.

In TRIAD, amyloid- β (^{18}F -AZD4694; 40–70 min post-injection) and tau (^{18}F -MK6240; 90–110 min post-injection) PET scans were acquired with a Siemens High Resolution Research Tomograph (Siemens Medical Solutions). PET data were reconstructed using the OSEM algorithm on a 4D volume (^{18}F -AZD4694, 3×600 s; ^{18}F -MK6240, 4×300 s). SUVR maps were generated using the cerebellar grey matter as reference region for ^{18}F -AZD4694 and the inferior cerebellar grey matter

for ^{18}F -MK6240. PET images were spatially smoothed to achieve a final resolution of 8 mm full-width at half-maximum (FWHM). T_1 -weighted images were acquired at 3 T for coregistration purposes and were corrected for non-uniformity and field distortion using an in-house pipeline.

Statistical analysis

The statistical software R (version 3.4.3) was used to perform statistical tests for demographic comparisons and data description. The R packages *lm* and *nlme* were used to perform linear models and linear mixed effect (LME)-based analyses, respectively. Cross-sectional data from ADNI and TRIAD were independently analysed with linear models. LME models were used only with longitudinal ADNI data to compare the progression in plasma NfL between cognitively unimpaired and cognitively impaired subjects; this model had plasma NfL as the dependent variable and included the main effects and the interaction between the independent variables time (days between baseline NfL and follow-up time points) and group and were adjusted for sex, age at initial NfL, with random intercepts accounting for the time correlation within subjects. Confidence intervals (95%) were determined based on the estimated fitted value across the distribution from 1000 simulations of the model (including all variations, except theta). Statistical significance was set at $P < 0.05$, two-sided.

The voxel-wise analysis also involved longitudinal and cross-sectional data. For the longitudinal data (ADNI only), two additional LME models were implemented. The first model addressed the longitudinal relationship between plasma NfL (independent variable) and voxel-based morphometry [VBM; dependent variable, adjusting for time (continuous), age at initial NfL, sex, scanner type (1.5 or 3 T) and the time between plasma collection and MRI, with a random intercept]. The second model addressed the longitudinal association between the interaction of plasma NfL (independent variable) with time in relation to the dependent variable, VBM [adjusting for time of assessment (0, 12, 24, 36 or 48 months), age, sex, scanner type (1.5 or 3 T)] and a correction factor for time gaps between plasma collection and MRI acquisitions. Voxel-based grey and white matter morphometric analyses were performed for cognitively unimpaired and cognitively impaired groups separately. Finally, voxel-wise linear models examined the cross-sectional associations between plasma NfL (predictor) and amyloid- β or tau PET (dependent variables). These models were first applied in ADNI and then subsequently reassessed in the TRIAD cohort. The linear models included corrections for either amyloid- β or tau (i.e. models with tau PET as the outcome were adjusted for global amyloid- β and vice versa), and age, sex and time interval between plasma and PET.

All voxel-wise analyses were performed using VoxelStats (Mathotaarachchi *et al.*, 2016) and findings were corrected for multiple comparisons using random field theory. Since we hypothesized that elevated plasma NfL would be associated with elevated amyloid- β and tau PET and decreased brain volume using VBM, one-tailed hypothesis tests with a type I error $\alpha < 0.05$ were performed.

For complete details on the statistical models and voxel-wise analyses, see the Supplementary material.

Table 1 Demographics and key characteristics of the ADNI and TRIAD cohorts

	ADNI			TRIAD		
	CU	CI	P-value*	CU	CI	P-value*
Subjects, n (%)	382 (33.2)	767 (66.7)	NA	74 (63.7)	42 (36.2)	NA
Age, years, mean (SD)	73.5 (6.9)	74.4 (7.8)	0.05	72.4 (6.2)	70.4 (8.5)	0.15
Males, n (%)	173 (45.3)	456 (59.5)	<0.001	24 (32.4)	21 (50.0)	0.09
Education, years, mean (SD)	16.6 (2.5)	15.9 (2.7)	<0.001	15.3 (4.2)	13.7 (3.8)	0.04
APOE ε4, n (%)	106 (27.7)	395 (51.6)	<0.001	25 (33.8)	17 (40.5)	0.10
MMSE, mean (SD)	29.0 (1.1)	26.4 (3.4)	<0.001	28.9 (1.3)	23.4 (5.3)	<0.001
Plasma NfL, ng/l	35.2 (16.7)	44.0 (22.2)	<0.001	27.3 (19.2)	34.5 (13.7)	0.01

MMSE = Mini-Mental State Examination; NA = not applicable.

*P-values were calculated comparing cognitively unimpaired (CU) and cognitively impaired (CI) subjects, within each cohort, using t-test for continuous variables and χ^2 test for categorical variables.

Data availability

Raw data are available for download upon request at <http://adni.loni.usc.edu>. Derived data supporting the findings of this study are available from the corresponding author on request.

Results

Demographic characteristics

A total of 1265 subjects were included in the present study (Table 1 and Supplementary material). From the ADNI cohort, 1149 subjects were included; of these, 215 had only baseline data available. The initial plasma NfL concentrations were on average higher in the cognitively impaired group compared to the cognitively unimpaired group ($t = 6.65$; $P < 0.0001$; Fig. 2A), adjusting for age and sex. Plasma NfL was highly associated with age ($t = 19.50$; $P < 0.0001$), but not with sex, education or APOE ε4 status

when adjusting for diagnosis. In the TRIAD cohort, comprising 116 participants with cross-sectional data, cognitively impaired participants had higher levels of plasma NfL ($t = 2.55$; $P = 0.011$; Fig. 2B), compared to cognitively unimpaired participants. Similar to ADNI, age had a large effect on plasma NfL ($t = 4.23$; $P < 0.0001$) but sex, education and APOE ε4 status (when adjusting for diagnosis), did not.

When using longitudinal data from ADNI, the LME analysis showed that plasma NfL differed between groups over time ($t_{groups} = 6.58$, $P < 0.0001$; Fig. 2C). However, no difference was found between the slopes [$t_{interaction} (groups \times time) = 1.40$; $P = 0.16$], indicating that the rate of increase in plasma NfL levels did not differ between groups (plasma NfL time-point correlations are presented in the Supplementary material). Comparison of plasma NfL concentrations in the cognitively unimpaired and cognitively impaired by amyloid-β status showed that amyloid-β-positive subjects had higher concentrations of

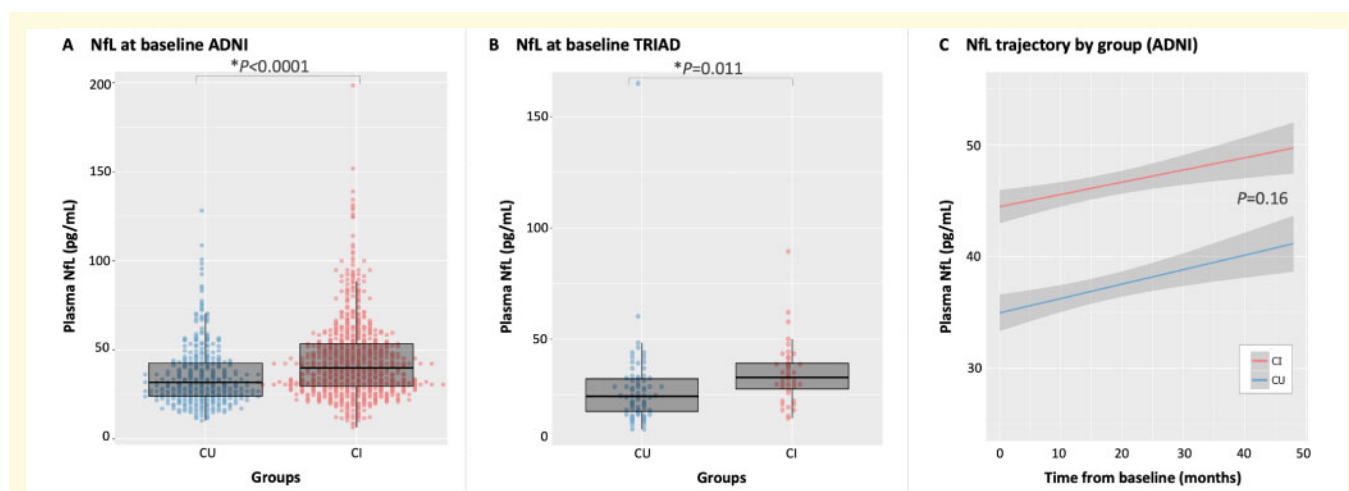


Figure 2 Plasma NfL. Findings from linear models (adjusted by age and sex) showed that differences in plasma NfL levels were seen between cognitively unimpaired (CU) and cognitively impaired (CI) subjects in both ADNI (A) and TRIAD (B) cohorts cross-sectionally. Cognitively impaired participants also showed higher plasma NfL concentrations compared to cognitively unimpaired participants (C) longitudinally, as shown by LME modelling.

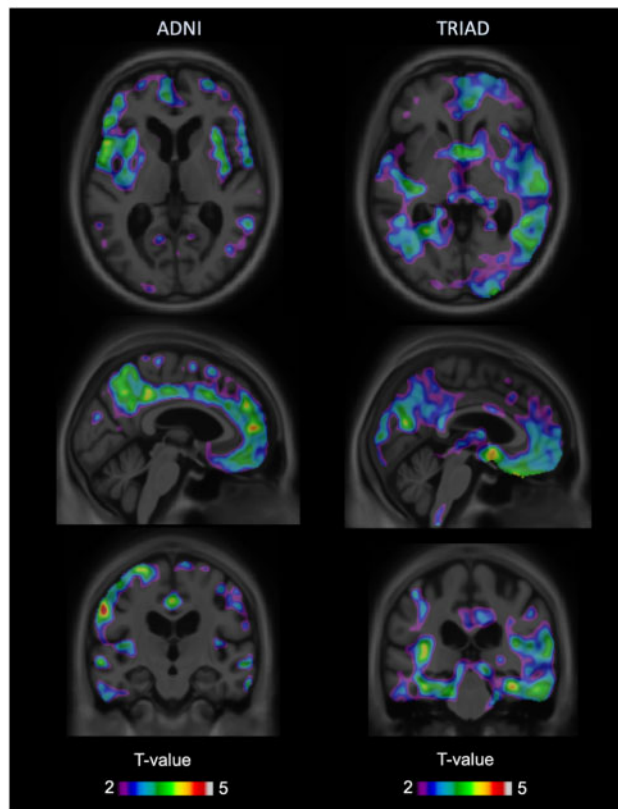
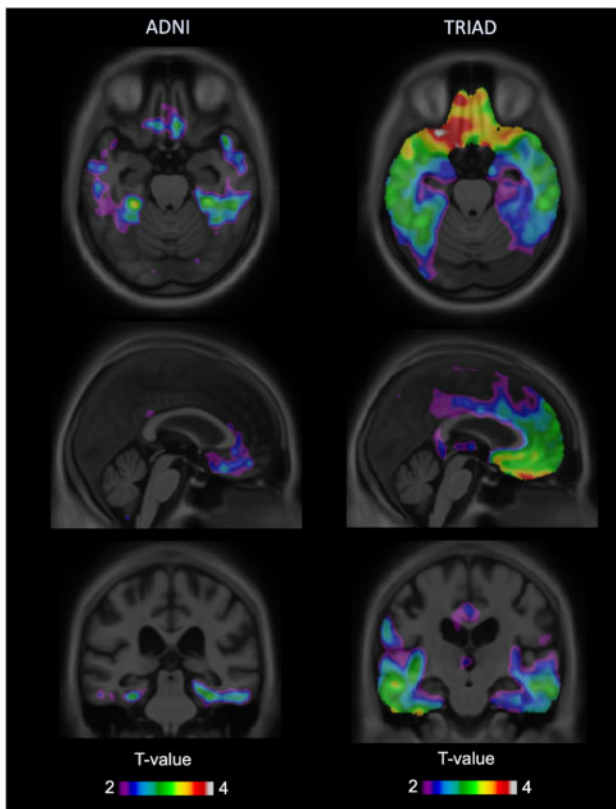
A Voxel-based associations between Amyloid PET and plasma NfL in CU subjects**B** Voxel-based associations between Tau PET and plasma NfL in CI subjects

Figure 3 Plasma NfL and PET biomarkers. T-statistical parametric maps (T-maps) superimposed on average structural MRI show brain regions where higher NfL levels were associated with higher ^{18}F -florbetapir (ADNI) and ^{18}F -AZD4694 (TRIAD) SUVRs in the cognitively unimpaired (CU) group (**A**). In the cognitively impaired (CI) group (**B**), T-maps also show brain regions where higher NfL levels are associated with higher ^{18}F -flortaucipir (ADNI) and ^{18}F -MK6240 (TRIAD) in cognitively impaired subjects. T-values that were significant after random field theory correction for multiple comparisons are indicated in the text.

plasma NfL cross-sectionally (Supplementary material) and a steeper rate of progression over time, compared to amyloid- β -negative subjects (Supplementary material).

Plasma NfL is associated with amyloid- β and tau in Alzheimer's disease-related brain areas

The cross-sectional voxel-wise analysis showed an association between plasma NfL and ^{18}F -florbetapir in cognitively unimpaired participants [Fig. 3A; ADNI, $t(103)$ and TRIAD, $t(67) > 3.21$, both $P < 0.05$]. These associations were seen primarily in the posterior cingulate/precuneus, parietal cortex, frontal and temporal cortices (peak t -values, along with their coordinates/exact P -values, and effect size information, are available in the Supplementary material). No significant associations were seen between plasma NfL and amyloid PET among cognitively impaired subjects.

In ADNI, ^{18}F -flortaucipir showed some association with plasma NfL in the cognitively impaired group [$t(79) > 3.19$, $P < 0.05$; Fig. 3B], although these results did not survive

multiple comparison correction. In the TRIAD cohort, tau load (^{18}F -MK6240) and plasma NfL were associated only in the cognitively impaired group [$t(35) > 3.34$, $P < 0.05$], showing strong associations in the frontal and temporal regions.

Plasma NfL is associated with grey matter atrophy in Alzheimer's disease-related brain areas in APOE $\epsilon 4$ carriers

The voxel-wise LME (adjusted for time and other covariates) demonstrated increases in plasma NfL levels associated with reduced grey matter volume in cognitively unimpaired [$t(1000) < -3.09$, $P < 0.05$; Fig. 4A] and cognitively impaired participants [$t(1969) < -3.09$, $P < 0.05$; Fig. 4B]. Associations in the cognitively unimpaired group were confined to small clusters in the frontal lobe and hippocampus. Among cognitively impaired participants, more widespread

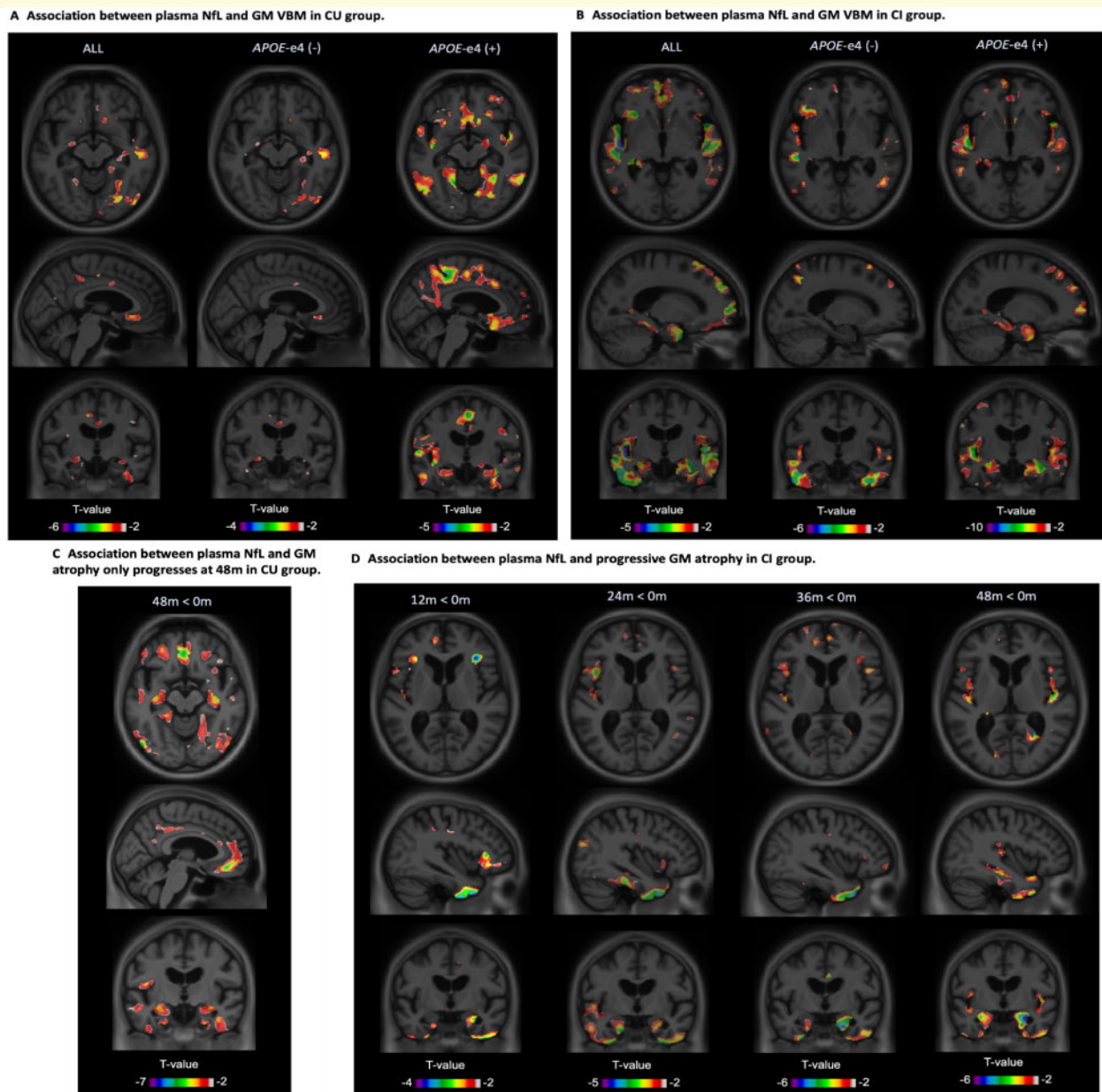


Figure 4 Plasma NfL and grey matter volume. T-statistical parametric maps (T-maps) superimposed on average structural MRI show brain regions where higher NfL levels were associated with reduced grey matter volume in cognitively unimpaired (CU; **A**) and cognitively impaired (CI; **B**) participants, subdivided by *APOE* $\epsilon 4$ status [carriers (+) or non-carriers (-)]. T-maps also show that, as compared to baseline, only at 48 months was there a reduction of grey matter (GM) volume associated with plasma NfL in cognitively unimpaired subjects (**C**), while in the cognitively impaired group differences were observed at each time point (**D**). T-values that were significant after random field theory correction for multiple comparisons are indicated in the text.

associations were seen in frontal and temporal cortices, as well as in the medial temporal lobe.

Given the above described association between plasma NfL and amyloid PET in cognitively unimpaired subjects, cognitively unimpaired and cognitively impaired groups were subdivided according to *APOE* $\epsilon 4$ status due to $\epsilon 4$ carriers being more likely to have amyloid- β accumulation (Morris *et al.*, 2010; Fleisher *et al.*, 2013). When doing so, a

clear distinction was seen between *APOE* $\epsilon 4$ carriers and non-carriers among cognitively unimpaired subjects, with findings localized in the frontal, posterior cingulate and temporal cortices of $\epsilon 4$ carriers only [$t(260) < -3.12$, $P < 0.05$] and no associations found in $\epsilon 4$ non-carriers. Among the cognitively impaired subjects, no clear differences were seen between *APOE* $\epsilon 4$ carriers and non-carriers, with both groups showing significant associations between plasma NfL

and grey matter volume [$t_{\text{carriers}(1009)} < -3.09$, $t_{\text{non-carriers}(951)} < -3.09$, $P < 0.05$], mainly in the temporal cortices. *APOE* $\epsilon 4$ carriers, however, appeared to have more medial temporal atrophy.

When examining the association between grey matter volume and plasma NfL over time (NfL \times time), cognitively unimpaired subjects only showed significant findings for the contrast baseline versus 48 months [$t(993) < -3.09$, $P < 0.05$; Fig. 4C], with results seen primarily in the frontal and temporal cortices. Cognitively impaired subjects, by contrast, showed a progressive reduction in grey matter volume associated with increased plasma NfL, predominantly in the temporal cortex [$t(1962) < -3.09$, $P < 0.05$; Fig. 4D].

Plasma NfL correlates with white matter atrophy

In addition to grey matter loss, increases in plasma NfL concentrations were accompanied by a reduction in white matter volume. Using voxel-wise LME (adjusted for time and other covariates), cognitively unimpaired participants showed significant associations between plasma NfL and white matter volume in the hippocampus, parietal and prefrontal cortices [$t(1005) < -3.09$, $P < 0.05$; Fig. 5A]; however, these findings were very focal. By contrast, widespread associations were seen within the cognitively impaired group [$t(1947) < -3.09$, $P < 0.05$]. When the LME tested the effect of NfL and time (interaction) on white matter, plasma NfL was found to associate with white matter volume in superior periventricular areas in the cognitively unimpaired group [$t(998) < -3.09$, $P < 0.05$; Fig. 5B]. Only at Month 36, however, was it possible to detect clear findings in temporal regions. In the cognitively impaired group, by contrast, these associations were seen at 12 months in temporal regions [$t(1940) < -3.09$, $P < 0.05$; Fig. 5C] and spread across the additional time points.

Discussion

The present study, to our knowledge, is the first to examine the associations between plasma NfL and whole brain imaging measures of amyloid- β , tau pathology and atrophy (grey and white matter). Consistent with previous work (Sjogren *et al.*, 2000; Mattsson *et al.*, 2017, 2019; Ashton *et al.*, 2019), we showed a linear increase in plasma NfL levels from cognitively unimpaired controls through MCI and Alzheimer's disease. Our main finding was that the associations between NfL, the aggregation of amyloid- β and tau, and brain atrophy, were disease stage-specific. Specifically, while a positive correlation was observed between plasma NfL and amyloid- β PET in cognitively unimpaired participants, NfL was found to correlate with tau PET in cognitively impaired patients. We also observed that NfL and grey matter atrophy associations, found in cognitively unimpaired and cognitively impaired individuals, were dependent on *APOE* $\epsilon 4$ status only in cognitively unimpaired individuals.

Importantly, these associations predominantly occurred in voxels within Alzheimer's disease-related brain areas. Despite longitudinal increases in plasma NfL, its association with grey matter volume loss was only evident at 48 months in cognitively unimpaired subjects, while in cognitively impaired subjects the association between NfL and grey matter volume progressively increased over 48 months. In cognitively unimpaired subjects, NfL was also associated with small foci of white matter atrophy in the anterior and posterior cingulate as well as the angular bundle, contrasting with a more global pattern of white matter atrophy in cognitively impaired subjects. In fact, the longitudinal associations between plasma NfL and white matter atrophy progressively involved the entire periventricular region in cognitively unimpaired subjects, in contrast to cognitively impaired subjects, where the more widespread findings observed at 48 months seemed to have propagated from the temporal lobe.

The observed relationship between plasma NfL and amyloid- β load among cognitively unimpaired subjects was in agreement with the existing literature. Although a negligible degree of neuronal injury has been reported in preclinical Alzheimer's disease (i.e. amyloid- β -positive cognitively unimpaired) (Mattsson *et al.*, 2017), our results corroborate the framework proposing that the initial neural injury in Alzheimer's disease pathophysiology, here indexed by plasma NfL, is linked to the accumulation of amyloid- β , rather than tau (Mielke *et al.*, 2019). Another study demonstrated that baseline plasma NfL among cognitively unimpaired subjects did not relate significantly to amyloid- β accumulation over a short follow-up interval, although increases in plasma NfL tracked increased signal from amyloid- β PET (Mielke *et al.*, 2019). In addition, the brain regions found here to be linking amyloid- β to neurodegeneration in cognitively unimpaired are frequently reported as having high levels of amyloid- β in Alzheimer's disease (Grothe *et al.*, 2017). This scenario would prove consistent with the topographical overlap we observed between areas showing significant associations between plasma NfL and amyloid PET (e.g. the temporal cortex and precuneus), and areas showing declines in grey matter volume. Similarly, one study has shown that the association between NfL and amyloid- β was also accompanied by declines in hippocampal volume and global cortical thickness and speculated that the observed association with amyloid PET may reflect Alzheimer's disease-related neurodegeneration (Mielke *et al.*, 2019). The observed association between plasma NfL levels and hippocampal white matter atrophy supports previous work showing that white matter integrity in this structure is affected early on in the course of Alzheimer's disease (Salat *et al.*, 2010), likely due to the accumulation of tau pathology (Jacobs *et al.*, 2018).

In contrast with the cognitively unimpaired group, plasma NfL associations were confined to tau PET among cognitively impaired subjects. While there are—to date—no other studies that have used tau PET when looking at plasma NfL in both cognitively unimpaired and cognitively impaired groups, a recent study in cognitively impaired subjects

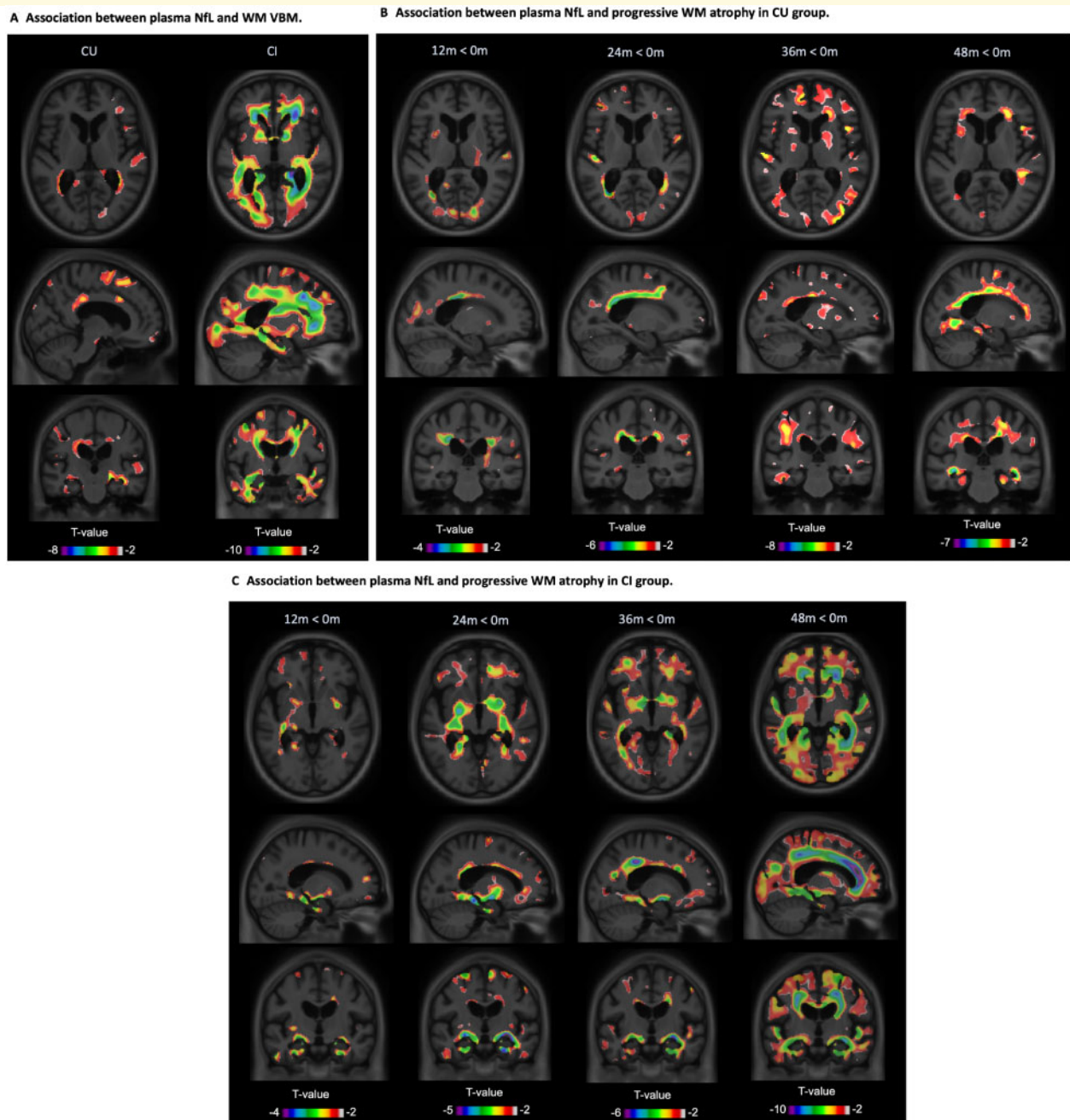


Figure 5 Plasma NfL and white matter volume. T-statistical parametric maps (T-maps) superimposed on average structural MRI show brain regions where higher NfL levels were associated with reduced white matter (WM) volume in cognitively unimpaired (CU) and cognitively impaired (CI) participants (A). T-maps also showed that, compared to baseline, there was a reduction of white matter volume associated with plasma NfL in cognitively unimpaired (B) and cognitively impaired groups (C) at each time point (D). T-values that were significant after random field theory correction for multiple comparisons are indicated in the text.

reported associations between plasma NfL and tau PET similar to those presented here (Thijssen *et al.*, 2020). In addition, previous work has shown plasma NfL to positively correlate with CSF phosphorylated tau (p-tau) over time in cognitively impaired subjects (Mattsson *et al.*, 2019). Similar findings have also been reported using NfL from cross-sectional plasma and CSF samples (Zetterberg *et al.*, 2016; Pereira *et al.*, 2017). The fact that the association was

specific to tau in the cognitively impaired group may reflect cognitively impaired subjects showing continued accumulation of tau pathology (McDade and Bateman, 2018), in contrast with amyloid- β deposition, which is thought to plateau during the symptomatic course of Alzheimer's disease (Jack *et al.*, 2013b). Voxel-wise associations between plasma NfL and tau PET differed between cohorts, however, with findings that did not survive multiple comparison correction in

ADNI, in contrast to robust positive associations within frontotemporal regions in the TRIAD cohort. This observed difference may relate to differing time intervals between plasma sampling and tau PET between cohorts. Although models accounted for this difference, this, combined with ^{18}F -florotau-cipir and ^{18}F -MK6240 differing in their sensitivity to tau aggregates (Hostetler *et al.*, 2016), may account for the observed discrepancy. In addition, as compared with ADNI, the TRIAD cohort included patients with more advanced disease stage, as evidenced by CDR ranges; this may have influenced our findings. Overall, however, the overlap of NfL-associated brain regions between cohorts and in comparison with previously reported findings (Thijssen *et al.*, 2020) suggests that the reported associations are valid.

The discrepancies in the association of plasma NfL with amyloid- β and tau PET in the cognitively unimpaired and cognitively impaired groups, as discussed above, are in line with the literature suggesting a pathophysiological model for Alzheimer's disease in which amyloid- β biomarkers become abnormal earlier than tau biomarkers (Jack *et al.*, 2013a). Both processes, however, are upstream from neurodegeneration. This may partially explain the differential association observed between plasma NfL and PET findings across groups. Importantly, however, our findings remain correlational at the cross-sectional level and cannot support any claims of causality.

Using VBM, plasma NfL was found to associate with both grey and white matter volume loss in cognitively unimpaired and cognitively impaired subjects. Using grey matter VBM data, although findings were limited when looking across all cognitively unimpaired subjects, analysis by *APOE* subgroups ($\epsilon 4$ carriers and non-carriers) showed significant declines in grey matter volume in temporal, posterior cingulate and orbitofrontal regions among $\epsilon 4$ carriers. As described above, these findings overlapped spatially with areas that showed a significant association between plasma NfL and amyloid- β imaging and may be due to *APOE* $\epsilon 4$ carriers showing enhanced amyloid- β deposition (Reiman *et al.*, 2009). Reinforcing this idea, we have shown in a recent study (Benedet *et al.*, 2019) that neurodegeneration in cognitively unimpaired subjects, indexed by reduced ^{18}F -fluorodeoxyglucose PET uptake, was only associated with NfL in amyloid- β -positive individuals, displaying regional associations similar to those described above. Longitudinally, only at 48 months, however, was the relationship between grey matter VBM and plasma NfL seen to progress. This suggests a temporal delay between the build-up of amyloid- β pathology and neuronal injury. As hypothesized, among cognitively impaired subjects, the coupling between NfL and grey matter loss was more pronounced, particularly within the temporal lobe, and showed a continuous increase across time points. Moreover, associated areas overlapped with those showing significant associations between plasma NfL and tau PET. These findings are consistent with neuroimaging studies showing progressive neuronal loss across the symptomatic phase of Alzheimer's disease (Jack *et al.*, 2004) and the co-localization of tau and

neurodegeneration (Benedet *et al.*, 2019). Not surprisingly, no notable difference was seen in the grey matter associations between cognitively impaired *APOE* $\epsilon 4$ carriers and non-carriers. As expected, at this disease stage the majority of the subjects showed evidence of amyloidosis and neurodegeneration, regardless of their *APOE* $\epsilon 4$ status.

In addition to the loss of cortical neurons, white matter injury (Migliaccio *et al.*, 2012) and the loss of cortico-subcortical connectivity (Delbeuck *et al.*, 2003) are features of Alzheimer's disease. In cognitively unimpaired and cognitively impaired subjects, the extent of white matter volume loss exceeded that of grey matter loss. This finding is also in agreement with early CSF studies showing an association between NfL and CT measures of white matter changes (Sjogren *et al.*, 2001). Since plasma NfL levels are understood to reflect damage to large-calibre myelinated axons (Ashton *et al.*, 2019), this would suggest that white matter damage may precede grey matter loss in Alzheimer's disease. Indeed, there is evidence to support the position that white matter abnormalities precede grey matter changes: CSF amyloid- β levels correlate with white matter lesions in cognitively normal elderly (Skoog *et al.*, 2018), and changes in CSF tau measures and amyloid- β_{1-42} have been shown to predict magnetic resonance-based measures of white matter integrity in cognitively unimpaired individuals at risk of Alzheimer's disease in the absence of effects on grey matter (Bendlin *et al.*, 2012). Moreover, soluble amyloid- β is toxic to oligodendrocytes (Lee *et al.*, 2004) and elevated in the white matter (Collins-Praino *et al.*, 2014). Further, while the neocortical tau pathology seen in Alzheimer's disease mainly affects grey matter, glial tangles are also seen in oligodendroglia (Ballatore *et al.*, 2007) and phosphorylated tau in grey matter has been shown to be associated with demyelination and white matter abnormalities in Alzheimer's disease (McAleese *et al.*, 2017). Amyloid- β and tau may thus first result in white matter damage, as reflected by increases in plasma NfL, which subsequently result in grey matter loss but with a spatiotemporally offset course.

This study has limitations. First, we did not account for vascular burden in our analyses, which may limit the interpretation of findings. In addition, the ADNI and TRIAD cohorts used for cross-sectional analyses differed in sample size, in the average time interval between plasma and PET measurements, and in the PET tracers used. While the difference in the time between plasma and PET was accounted for in statistical models, this may have affected the comparability of findings between cognitively impaired groups. Furthermore, even though plasma NfL measurements for both ADNI and TRIAD were performed in the same laboratory using the same in-house assay, different batches of reagents were used in these studies, resulting in plasma NfL levels being higher in ADNI compared to TRIAD; this difference was confirmed by evaluating the internal controls used, which were the same in both analyses. Despite these differences, however, it is important to stress that results were quite similar between cohorts. Moreover, a larger sample size combined with the inclusion of non-Alzheimer's disease

subjects and longitudinal PET data would have allowed us to have a detailed investigation of the ability of plasma NFL to capture the neurodegenerative effects of both amyloid- β and tau, as well as aspects of neuronal damage that are non-related to either proteinopathy. Lastly, though not the aim of this study, the current study design precludes us teasing apart the exact contributions of amyloid- β , tau and neurodegeneration to plasma NFL levels.

In conclusion, the present findings suggest that plasma NFL may prove a marker of early amyloid- β -related neuronal injury in Alzheimer's disease at its presymptomatic stage, being in turn more closely related to tau-mediated neurodegeneration during the symptomatic course of the disease. Further, the association of elevated plasma NFL to widespread grey and white matter loss provides further evidence supporting the use of NFL as marker of progressive neuronal damage.

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Competing interests

K.B. has served as a consultant or on advisory boards for Alector, Alzheon, CogRx, Biogen, Lilly, Novartis and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, all unrelated to the submitted work. H.Z. has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, all unrelated to the submitted work.

Supplementary material

Supplementary material is available at *Brain* online.

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