Associations between plasma neurofilament light, in vivo brain pathology, and cognition in non-demented individuals with autosomal-dominant Alzheimer's disease

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Search terms: Alzheimer's disease, presenilin-1, preclinical, pathology, NfL, biomarkers

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ABSTRACT

Background: Neurofilament light (NfL) is a promising biomarker of neurodegeneration. Our recent work with the Colombian kindred with autosomal-dominant Alzheimer's disease demonstrated that plasma NfL levels begin to differentiate carriers from non-carriers 22 years before the onset of mild cognitive impairment (MCI) at an estimated median age of 44. Here, we examined whether plasma NfL was associated with markers of brain pathology (amyloid-β and tau) and cognitive performance in non-demented members of the kindred.

Methods: Twenty-four *PSEN1* E280A carriers were included in this study (18 cognitively-unimpaired, 6 with MCI) and 19 non-carriers. Participants underwent PiB-PET, Flortaucipir-PET, blood sampling, and cognitive testing. Multiple regressions, adjusted by age, were used to examine how plasma NfL was associated with: mean cortical amyloid-β; a tau composite comprising the amygdala, entorhinal, inferior temporal and lateral occipital cortices; the CERAD word list delayed recall; and the Mini Mental State Examination (MMSE).

Results: Mutation carriers exhibited higher plasma NfL levels than non-carriers (p=.001). In mutation carriers, higher NfL levels were related to greater tau burden (p=.017), and worse memory (p<.001) and MMSE (p=.004) scores, but not amyloid-β. When we adjusted for age, a proxy of disease progression in this kindred, elevated plasma NfL levels were only correlated with worse memory (p=.001).

Conclusions: Findings support an association between plasma NfL and memory, beyond the effects of age, as well as with tau in individuals at genetic risk to develop dementia. Plasma NfL

may be useful for selecting participants and tracking treatment response in clinical trials of AD-modifying drugs.

INTRODUCTION

There is an urgent need for widely available and inexpensive biomarkers of Alzheimer's disease (AD) that can be used in clinical trials to evaluate the efficacy of disease-modifying drugs. One promising candidate is neurofilament light (NfL), a sensitive marker of early neural injury and axonal degeneration that has been shown to be elevated in preclinical AD [1-4]. We recently reported that plasma NfL levels were significantly elevated in individuals from a Colombian kindred with autosomal-dominant AD (ADAD) who are nearly certain to develop dementia[5]. Plasma NfL levels subtly started diverging between groups approximately 22 years before the kindred's estimated median age of clinical symptom onset[5]. Our data also showed that higher baseline levels of plasma NfL predicted greater cognitive decline in the preclinical stage of the disease. These findings are in line with studies from the Dominantly Inherited Alzheimer's Network (DIAN), which have reported that serum NfL levels can distinguish mutation carriers from non-carriers approximately 7 to 16 years before the expected age of clinical symptom onset [6,7]. The disparity between cohorts in the estimated time in which NfL levels begin to differentiate carriers from non-carriers may be due to sample characteristics. Specifically, the DIAN studies a heterogeneous sample composed of families with distinct mutations in different genes, whereas the Colombian kindred is a homogenous sample of individuals with a single mutation (E280A) in the Presenilin-1 (*PSEN1*) gene who share a similar clinical profile. Notwithstanding, together these studies suggest that blood-based NfL levels are sensitive to early neuronal degeneration in ADAD.

These findings are further supported by recent studies examining the relationship between blood-based NfL levels and other brain imaging markers of neurodegeneration in individuals in the preclinical stage of AD. Specifically, cross-sectional studies have reported that greater

plasma NfL levels are related to reduced hippocampal atrophy, precuneus cortical thickness and whole-brain volume, as well as reduced metabolism in those same regions[4,6-8]. Similarly, longitudinal studies in both familial and sporadic AD have shown that higher baseline NfL levels in the blood are associated with greater subsequent rates of reduction in precuneus cortical thickness, faster white matter intensity changes, and greater decline in glucose metabolism and cognitive performance [6,9-11].

More recent studies have investigated the association between plasma NfL levels and markers of AD-related pathology in the cerebrospinal fluid (CSF), blood (serum and plasma), and *post-mortem* tissue. One of these studies showed that symptomatic ADAD mutation carriers with greater serum NfL levels had significantly higher total and phosphorylated tau levels in the CSF, compared to non-carriers[12]. Consistent with these findings, higher plasma NfL levels have been related to increased neurofibrillary tangle accumulation in *post-mortem* tissue of older adults with a clinical diagnosis of AD dementia, and greater severity of the disease[13]. Further, a population-based longitudinal study found that elevated plasma NfL and low plasma amyloid-β_{42} levels, individually and in combination at baseline, and not total tau, significantly predicted risk for progression to AD dementia in older adults[14]. Altogether, the few available studies have provided some insight into the utility of blood-based NfL for tracking AD progression. However, very little is known about whether plasma NfL could be useful for tracking AD pathology accumulation in the living brain (especially neurofibrillary tau burden) in non-demented individuals at high risk for AD. Understanding how plasma NfL is related to AD pathology can inform future clinical trials, assist with participant selection and track disease progression, and treatment outcomes.

In this study, we sought to test whether baseline levels of plasma NfL were associated with brain markers of cortical amyloid-β and tau pathology burden, as well as cognition, in *PSEN1*

E280A cognitively-unimpaired carriers and carriers with mild cognitive impairment (MCI) from the world's largest ADAD kindred. The disease in *PSEN1* E280A carriers is estimated to progress to MCI at a median age of 44 years (95% confidence interval: 43-45) and dementia at the age of 49 (95% confidence interval: 49-50) [15]. Mutation carriers also have a well-characterized disease trajectory with cortical amyloid-β accumulation beginning over a decade before the onset of MCI, elevated tau burden in medial temporal lobe regions (e.g. entorhinal cortex and inferior temporal gyrus) an average of six years before the onset of MCI as measured by positron-emission tomography (PET) [15], and cortical atrophy an average of six years before clinical symptom onset [15-17]. We hypothesized that higher plasma NfL concentration would be associated with greater AD pathology burden, including mean cortical amyloid-β and tau burden in an aggregate of regions that are vulnerable to early pathology accumulation[15,18,19]. We also hypothesized that higher plasma NfL concentration would be related to worse cognitive performance in carriers with and without cognitive impairment.

METHODS AND MATERIALS

Study design and participants

Baseline plasma NfL concentrations were characterized in 24 *PSEN1* E280A mutation carriers (18 cognitively-unimpaired mutation carriers and 6 mutation carriers with MCI), and 19 age- and education-matched non-carriers from the same kindred who are enrolled in the Massachusetts General Hospital (MGH) COLBOS (Colombia-Boston) longitudinal biomarker study. Participants were recruited from the Alzheimer's Prevention Initiative registry of familial AD, which currently includes more than 6,000 living members of the kindred and approximately 1,200 mutation carriers[20]. Those with a diagnosis of dementia or with a significant medical, psychiatric, or neurological disorder (e.g., stroke, seizures, substance abuse, and other disorders that affect

motor, visuospatial or cognitive abilities) were excluded from this study. Participants and raters were not informed of the participants' genetic test results.

The study was approved by both the institutional ethics review boards of the University of Antioquia in Medellín, Colombia and the MGH in Boston, MA. All participants provided written informed consent before participating in any procedures.

Clinical and Cognitive Assessments

Clinical assessments were performed at the University of Antioquia in Medellín, Colombia. Participants underwent a clinical interview and were administered the Mini Mental State Examination (MMSE) [21], a Spanish version of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) word list test, which has been adapted for this Colombian population [22,23], and the functional assessment staging test (FAST) [24]. In the CERAD word list delayed recall, participants were asked to recall as many words as they could remember from a previously learned list (10 items) after a 10-minute delay.-Testing was conducted in Spanish by neuropsychologists or by psychologists trained in neuropsychological assessment. Neurological examinations were performed by a neurologist or by a physician trained in the assessment of neurodegenerative disorders.

Imaging acquisition and processing

All participants in this study travelled from Colombia to Boston (USA) and underwent amyloid and tau PET imaging, as well as MRI at the MGH.

11C Pittsburgh compound B and [F18] flortaucipir PET

As reported previously [15], 11C Pittsburgh compound B (PiB) PET was acquired with a 8.5 to 15 mCi bolus injection followed immediately by a 60-minute dynamic acquisition in 69 frames

(12x15 seconds, 57x60 seconds). [F18] Flortaucipir (FTP) was acquired between 80 and 100 minutes after a 9.0 to 11.0 mCi bolus injection in 4 separate 5-minute frames.

11C PiB PET data were expressed as the distribution volume ratio (DVR) with cerebellar grey as reference tissue; regional time-activity curves were used to compute regional DVRs for each region if interest (ROI) using the Logan graphical method applied to data obtained between 40 and 60 minutes after injection[25]. 11C PiB retention was assessed using a large cortical ROI aggregate that included frontal, lateral temporal and retrosplenial cortices as described previously [26].

[F18] FTP specific binding was expressed in FreeSurfer ROIs as the standardized uptake value ratio (SUVR) to cerebellum, similar to a previous report [27]. The spatially transformed SUVR PET data was smoothed with an 8mm Gaussian kernel to account for individual anatomic differences[28]. SUVR values were represented graphically on vertices at the pial surface. A tau summary metric was calculated by averaging regional SUVRs of the entorhinal cortex, amygdala, lateral occipital cortex, and inferior temporal cortex, as previously reported [15,18,19,29]. PET data were not partial volume corrected.

Genotyping

Genomic DNA was extracted from blood by standard protocols, and *PSEN1* E280A characterization was done at the University of Antioquia using methods previously described [30]. Genomic DNA was amplified with the primers PSEN1-S 5′ AACAGCTCAGGAGAGGAATG 3′ and PSEN1-AS 5′ GATGAGACAAGTNCCNTGAA 3′. We used the restriction enzyme BsmI for restriction fragment length polymorphism analysis. Each participant was classified as a *PSEN1* E280A carrier or non-carrier.

Plasma NfL assay

Plasma was collected in the morning (non-fasting collection). Three aliquots of 1ml were collected. Samples were stored at -80˚C. For NfL analysis, one plasma aliquot was shipped on dry ice to the Clinical Neurochemistry Laboratory at Sahlgrenska University Hospital, Mölndal, Sweden. NfL concentration was measured using an in-house Single molecule array (Simoa) assay, as previously described in detail (manufacturer: Quanterix, Billerica, MA)[31]. Measurements were performed by board-certified laboratory technicians who were blinded to clinical data, genetic status, and demographic characteristic.

Statistical Analyses

We used independent samples t-tests and Chi-square to test group differences in demographic and clinical variables. We conducted linear regressions to examine the association between plasma NfL and markers of pathology, and cognition. Specifically, we tested the cross-sectional associations between plasma NfL with the following variables: mean cortical amyloid- β; tau summary measure; the MMSE; and the CERAD word list delayed recall score. Further, as age in *PSEN1* E280A mutation carriers is predictive of disease progression, we also conducted multiple regressions, with age as a covariate. We used a univariate analysis of variance to examine group differences in NfL and cognitive performance with age as a covariate. Analyses used a family-wise significance threshold of $p<0.05$ and were performed using statistical software (SPSS V.24.0; SPSS Inc, Chicago, Illinois, USA). We used Cohen's *d* to calculate effect sizes and the Bonferroni correction to account for multiple comparisons.

In follow-up analyses, we carried out an exploratory whole-brain analysis examining the relationship between plasma NfL level and amyloid and tau pathology burden in all

mutation carriers. Regions were $p < 0.01$ after cluster size correction for multiple comparisons (minimum cluster extent k=100mm2).

RESULTS

Demographic and neuropsychological data

Demographic and neuropsychological data are presented in Table 1. Mutation carriers and non-carriers did not significantly differ in age, education, or sex. Compared to non-carriers, mutation carriers performed significantly worse than non-carriers in the CERAD word list delayed recall (F (1,41) =10.95, p=.002, d=1.14) and the MMSE (F (1,41)= 5.72, p=.021, d=0.90).

Plasma NfL levels in carriers and non-carriers

As expected, we found that mutation carriers had greater levels of plasma NfL relative to non-carriers (F (1,41)=14.73, p<.001, d=0.81) (Figure 1A). Higher plasma NfL levels were associated with greater age in carriers (B=0.389, p=.003, CI [0.149,0.629]), such that those who had higher NfL levels were older and, hence, closer to the median age of onset of MCI in this cohort (Figure 1B). There were no significant relationships between age and plasma NfL in non-carriers.

Association between plasma NfL levels, mean cortical Aβ, and regional tau

Amyloid-β and tau burden for each group are presented in Table 2. Consistent with what we previously reported[15], carriers had greater levels of amyloid-β (F (1,41)=69.04, p<.001, d=2.39) and tau pathology (F (1,41)=13.94, p=.001, d=1.38), compared to non-carriers. Plasma NfL levels were not related to mean cortical amyloid-β (p=.137), even after controlling for age (p=.170) (Figure 1C). In contrast, NfL levels were significantly related to the tau summary measure (B=0.026, p=.017, CI [0.005,0.047]). This association with tau did not survive when adjusted by age (p=.966) (Figures 1D). No associations between NfL and PET markers were observed in the non-carrier group.

We also examined the relationship between plasma NfL levels and brain pathology in the whole brain within mutation carriers. Consistent with findings using the summary measures, greater plasma NfL levels were related to higher tau burden in the precuneus and temporal lobe regions, including the entorhinal cortex (Figure 3). There were no associations between NfL and amyloid-β burden in mutation carriers.

Association between plasma NfL levels and cognition

In mutation carriers, higher plasma NfL levels were related to worse CERAD word list delayed recall scores (B=-0.607, p<.001, CI [-0.820,-0.394]) (Figure 2A) and MMSE scores (B=-0.475, p=.004, CI [-0.782,-0.169]) (Figure 2B). The association between plasma and memory performance remained significant after controlling for age (B=-0.376, p=.001, CI [-0.574, -0.178]), amyloid-β (B=-0.527, p<.001, CI [-0.726, -0.329]), and tau burden (B=-0.408, p<.001, CI [-0.571, -0.245]). Plasma NfL levels were associated with MMSE scores when adjusting for amyloid-β (B=-0.373, p=.016, CI [-0.668, -0.078]), but not for age (p=.188) or tau burden (p=.111). Plasma NfL levels were also not associated with cognitive performance in non-carriers.

DISCUSSION

This study examined the associations between plasma NfL levels, *in vivo* amyloid-β and tau pathology burden, and cognitive performance in non-demented ADAD mutation carriers who will develop dementia with virtually 100% certainty. As we previously reported, amyloid-β begins to accumulate in the brain of *PSEN1* E280A carriers in their late 20s, 15 to 20 years before clinical onset, and regional tau pathology is evident 5 to 10 years before dementia onset [15]. Whereas PET imaging has been proven to be valuable for the early identification of AD-related pathology, blood-based biomarkers of AD have gained increasing attention given their potential diagnostic value, accessibility, and utility for tracking disease progression and monitoring treatment

response. NfL, in particular, has been proposed as a promising biomarker of early neuronal injury, axonal degeneration, and synapse loss, as it has been shown to distinguish individuals at risk for AD dementia many years before clinical onset. In fact, we recently reported that plasma NfL levels began to distinguish *PSEN1* mutation carriers from non-carriers nearly 22 years before expected symptoms onset, and were strongly correlated with cognitive decline[5]. Yet, very little is known about how plasma NfL levels relate to *in vivo* AD pathology and cognition in individuals with ADAD who will go on to develop dementia.

Our results showed that compared to age-matched non-carriers, non-demented carriers with higher plasma NfL levels had greater tau burden, but not greater amyloid-β burden. However, plasma NfL levels were not associated with mean cortical amyloid-β burden or tau in an aggregate of regions of interest when covarying for age, a proxy of disease progression in this kindred. In contrast, we found that greater plasma NfL levels were related to worse memory, even beyond the effects of age. Contrary to what we previously reported [5], plasma NfL levels were not associated with age in non-carriers, which may be due to the limited age range in the current sample relative to the much larger dataset in our previous report with plasma NfL and cognitive data (but no PET imaging).

To date, the relationship between tau pathology and NfL in AD has only been reported in CSF, *post-mortem* tissue, and blood. Specifically, studies have found that elevated blood-based NfL levels are associated with greater CSF total and phosphorylated tau levels in symptomatic carriers of an ADAD mutation[12], and with greater neurofibrillary tangles in *post-mortem* tissue of older adults with AD dementia[13], but not plasma tau[32]. While no study to our knowledge has reported on the relationship between plasma NfL levels and PET tau in AD, one recent study[33] showed that in five of ten veterans with blast injuries who displayed high levels of tau binding also exhibited elevated plasma NfL levels. This suggests a link between plasma NfL and

aggregated neurofibrillary tangles measured by [F18] FTP PET. In our study, we found that plasma NfL did not relate to tau burden after controlling for age, raising the possibility that perhaps plasma NfL levels are not the best predictor of neurofibrillary tangles measured by PET in the very early stages of preclinical ADAD, when individuals have limited tau accumulation.

While prior studies in ADAD have reported a relationship between serum NfL levels and PET amyloid-β in symptomatic mutation carriers, we did not find an association between plasma NfL and amyloid-β burden [6]. However, our data show that plasma NfL levels can predict memory functioning beyond the effects of age, amyloid-β and tau pathology burden. As such, plasma NfL could be a very valuable tool to predict who is at high risk for AD dementia.

The current study has multiple strengths. First, we did not rely on presenting symptoms or cognitive data to infer whether individuals will go on to develop dementia. Instead, we examined blood-based NfL levels in a group of individuals who have a well-characterized clinical trajectory with MCI starting at a median age of 44 years and dementia at 49 years [15,22,34]. Studying ADAD provides a unique opportunity to study biomarkers of AD in the preclinical stage, as we can estimate how far mutation carriers are from the clinical symptom onset based on the mutation that they carry. In addition, we examined *in vivo* amyloid-β and tau pathology using PET imaging, which is considered the gold standard for quantifying and examining brain pathology in AD. Additionally, to our knowledge, this is the first study to assess how plasma NfL levels relates to the disease continuum by examining the two pathologies that characterize AD *in vivo*. Mutation carriers were also young and otherwise healthy, which minimizes potential confounding variables that are more common in advanced age and contribute to cognitive decline (e.g., cardiovascular disease). Finally, the nearly homogeneous clinical profile of mutation carriers allows us to infer how NfL levels may change as the disease progresses, supporting the utility of this blood-based biomarker for tracking disease progression.

The present study also has limitations which must be discussed. First, our sample size is relatively small compared to other studies of AD and cognitive aging. However, individuals with these mutations are relatively rare and all our participants had a single mutation (*PSEN1* E280A), which makes our sample highly homogeneous compared to other cohorts, and one of the larger single mutation ADAD samples with NfL and PET imaging. More research is also needed to examine whether our findings in ADAD generalize to preclinical late-onset sporadic AD. We are currently conducting the first longitudinal biomarker study with this kindred, which will provide greater insight into how annual change in plasma NfL levels relates to *in vivo* pathology burden and cognitive decline over time.

Taken together, our findings suggest that higher plasma NfL is associated with markers of brain pathology and worse cognitive performance in *PSEN1* E280A mutation carriers who are still years away from their estimated age of dementia onset. These results support the potential value of plasma NfL for tracking disease progression and monitoring treatment response in clinical trials of disease-modifying drugs for AD.

ACKNOWLEDGEMENTS

The authors thank the Colombian families with autosomal dominant Alzheimer's disease for contributing their valuable time and effort, without which this study would not have been possible. We also thank Francisco Piedrahita, Alex Navarro, and Claudia Ramos from Grupo de Neurociencias, Universidad de Antioquia in Medellín, Colombia, as well as Olivia Hampton, Heirangi Torrico-Teave, Jairo Martínez, and Diana Munera from the Massachusetts General Hospital in Boston, MA for helping coordinate visits to Boston and assisting with data collection and processing.

CONFLICTS OF INTEREST AND FUNDING SOURCES

Dr. Guzmán-Vélez was supported by the NIA K23AG061276 and NIH Research Supplement for Diversity (DP5OD019833). Dr. Quiroz was supported by grants from the NIH Office of the Director (DP5OD019833), the National Institute on Aging (R01 AG054671], the Alzheimer's Association, and Massachusetts General Hospital ECOR (1200-228010 and 1200-228767). Mr. Fox-Fuller reports NRSA support from the National Institute on Aging (1F31AG06215801A1). Dr. Vila-Castelar is supported by a grant from the Alzheimer's Association (2019A005859). Dr. Pardilla-Delgado is supported by the NHLBI 3T32HL007901-19S1. Dr. Zetterberg is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#681712) and Swedish State Support for Clinical Research (#ALFGBG-720931) and the UK Dementia Research Institute at UCL. He 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 (outside submitted work). Drs. Arboleda-Velasquez was supported by a grant co-funded by the National Institute on Aging and the National Institute of Neurological Disorders and Stroke (RF1 NS110048). Dr. Lopera was supported by an Anonymous Foundation, and the Administrative Department of Science, Technology and Innovation (Colciencias Colombia;111565741185). Drs. Reiman and Lopera are principal investigators of the Alzheimer's Prevention Initiative (API) Autosomal Dominant AD Trial, which is supported by NIA, philanthropy, Genentech, and Roche. Dr. Gatchel is supported by NIH/NIA (K23 AG058805-01), Alzheimer's Association Clinical Fellowship (AACF_16-440965), and the BrightFocus Foundation Fellowship (ADRF A2016434F), and the Massachusetts General Hospital Rappaport Fellowship. Dr. Reiman reports grants from National Institute on Aging (R01 AG031581, P30 AG19610), Banner Alzheimer's Foundation and the NOMIS Foundation during the conduct of the study. He reports receiving personal fees

as a Scientific Advisor to Roche Diagnostics (travel expenses only), MagQ, Avid Radiopharmaceuticals and is a share-holding co-founder of ALZPath, outside the submitted work. In addition, he is the inventor of a patent issued to Banner Health, which involves the use of biomarker endpoints in at-risk persons to accelerate the evaluation of Alzheimer's disease prevention therapies and is outside the submitted work. Dr. Blennow has served as a consultant or at advisory boards for Axon, Biogen, CogRx, Lilly, MagQu, Novartis and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg. Dr. Sperling receives research support for NIH grants P01AG036694, P50AG005134, 2009-2020, and U19 AG10483, as well as from Eli Lilly (clinical trial) and the Alzheimer's Association. She is a site principal investigator or coinvestigator for Avid, Bristol-Myers Squibb, Pfizer, and Janssen Alzheimer Immunotherapy clinical trials. She receives travel funding and honoraria from AC Immune, Janssen, and Roche. She consults for Biogen, Roche, AC Immune, Eisai, Takeda, Neurocentria, and Janssen. Spouse consults for Novartis, AC Immune and Janssen. Dr. Johnson has provided consulting services for Novartis, Biogen, and Eli Lilly, received support from a joint NIH-Lilly-sponsored clinical trial (A4 Study - U19AG10483), and received research support from NIH grants R01 AG027435, P50 AG00513421, AG036694, R01 AG046396, R13 AG042201174210, U19AG10483, and U01AG024904, as well as the Alzheimer Association and Marr Foundation. All other co-authors have no conflicts or disclosures relevant to the manuscript.

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Table 1. Demographic and neuropsychological data

	Non-carriers (n=19)	Mutation Carriers (n = 25) *M (SD)*	*p*
Age (years)	36.36 (5.18)	38.76 (5.98)	.535
Education	11.40 (3.95)	9.25 (4.68)	.200
Sex (f/m)	8/11	15/10	.361
MMSE	29 (1)	26.72 (3.43)	.021
FAST			
Stages 1-2	19	19	
Stages >2	0	6	
CERAD delayed recall	7.89 (1.20)	5.16 (3.15)	.002

Note. M = Mean; SD = Standard Deviation; MMSE=Mini Mental State Exam; FAST=Functional Analysis Screening Tool (stages 1 and 2=cognitively normal; stages >2=symptomatic); CERAD= Consortium to Establish a Registry for Alzheimer's Disease neuropsychological battery. Differences between cognitively-unimpaired mutation carriers and non-carriers were calculated using independent samples t-test (for age and education) and univariate analysis of variance controlling for age for the other variables.

Table 2. Measures of neurodegeneration, amyloid and tau pathology

	Non-carriers (n=19)	Mutation Carriers (n = 25) *M (SD)*	*p*
Neurofilament Light (pg/mL)	4.65 (2.10)	8.82 (4.01)	<.001
11C PiB PET (DVR)	1.04 (0.02)	1.33 (0.17)	<.001
Tau Summary Measure (SUVR)	1.04 (0.08)	1.25 (0.20)	.001

Note. M = Mean; SD = Standard Deviation; pg/mL = picograms per millilitre; DVR = distribution volume ratio; SUVR = standardized uptake value ratio; PiB = Pittsburgh Compound B. Differences between mutation carriers and non-carriers were calculated using using independent samples t-test (for age and education) and univariate analysis of variance controlling for age for the other variables.

Figure 1. Plasma NfL levels and pathology in mutation carriers and non-carriers. pg/mL = picograms per milliliter. (A) Mutation carriers (red) had significantly higher levels of plasma NfL compared to non-carriers (black). Graph depicts the mean and standard deviation for each group. (B) Black circles represent raw data for non-carriers, red circles represent cognitively-unimpaired carriers, and orange rectangles represent carriers with MCI. Lines represent the best fit line for each group and shadowed areas the confidence intervals. In mutation carriers only, higher plasma NfL levels were associated with higher age. (C, D) Plasma NfL levels were related to tau burden but not cortical amyloid-β without covarying for age.

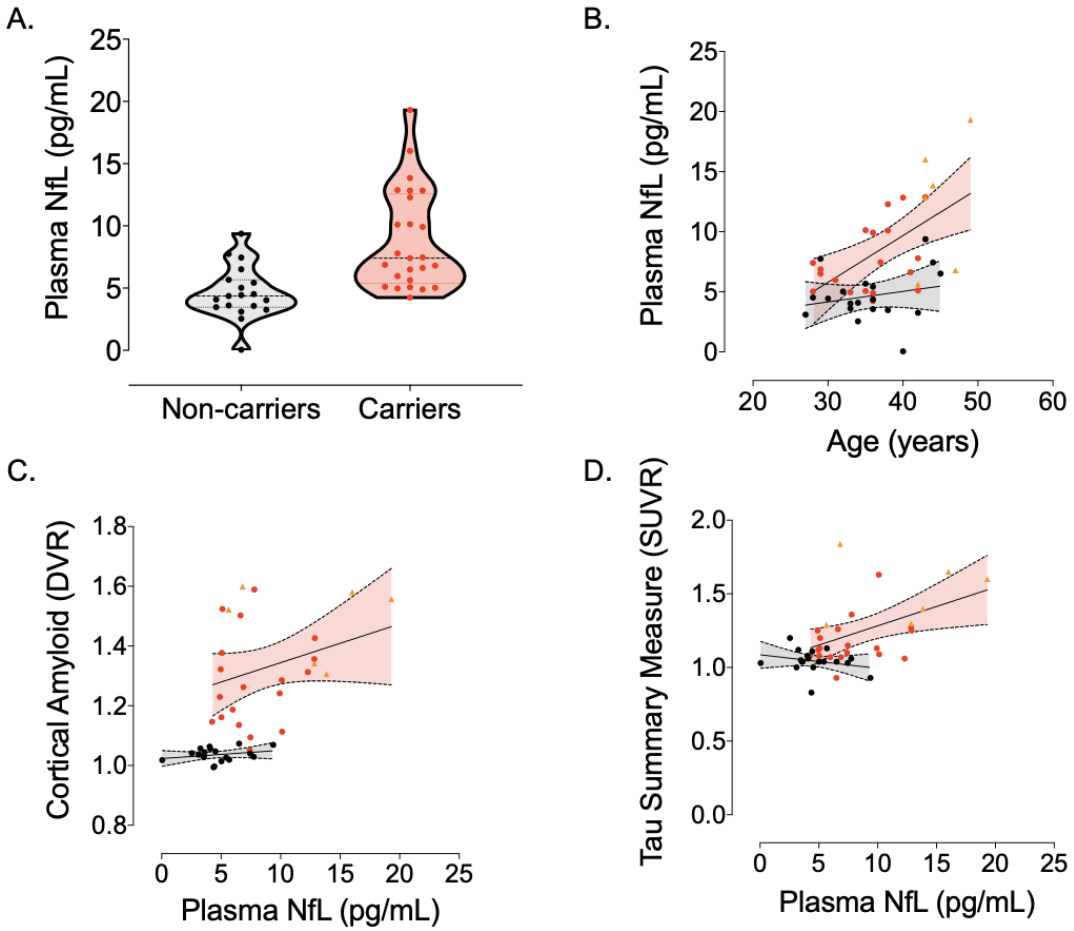

Figure 2. Plasma NfL levels and cognition in mutation carriers and non-carriers. Black circles represent raw data for non-carriers, red circles represent cognitively-unimpaired carriers, and orange rectangles represent carriers with MCI. Lines represent the best fit line for each group and shadowed areas the confidence intervals. Higher NfL levels in mutation carriers were related to lower scores on the CERAD word delayed recall and the MMSE. Only word delayed recall remained significant after adjusting for age.

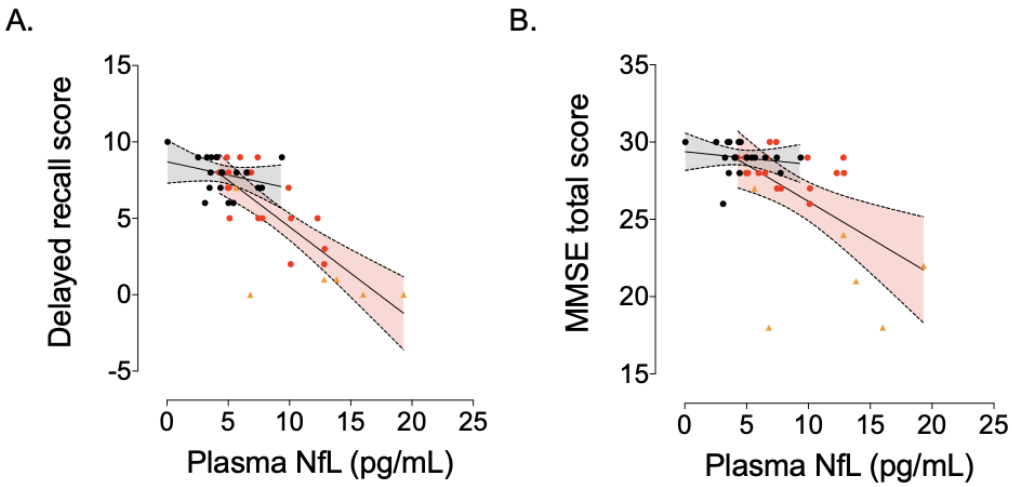

Figure 3. Whole-brain analysis of the relationship between plasma NfL levels and tau pathology. Higher plasma NfL concentration was related to 18F FTP-binding in the precuneus and temporal regions, including the entorhinal cortex, in mutation carriers. Regions shown are $p < 0.01$ after cluster size correction for multiple comparisons (minimum cluster extent k=100mm2).