ARTICLE

Plasma neurofilament light reflects more severe manifestation of Alzheimer's disease in men

Xiaoqin Cheng[1,6], Zhenghong Wang[2,6], Kun He[3,6], Yingfeng Xia[4,6], Ying Wang[5], Qihao Guo[5], Fang Xie[3]✉ and Peng Yuan[2]✉

Plasma neurofilament light (NfL) protein is a promising non-invasive biomarker for detecting neuronal damage in Alzheimer's disease (AD). However, its clinical utility is limited by the lack of standardized threshold values. Sex is an important factor that should be considered when setting these thresholds, but only a few studies have examined sex differences in plasma NfL levels in AD, with inconsistent findings. Even fewer have explored whether sex influences the relationship between plasma NfL levels and disease severity. To investigate this, we first analyzed data from 860 participants in the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. Linear regression models were used to assess sex differences in the correlation between plasma NfL levels, cognitive deficits, and neuroimaging metrics. A Cox model with bootstrap resampling was used to evaluate sex differences in dementia risk, calculating the hazard ratio for men versus women for a given increase in plasma NfL. Our results showed that, compared to women, men with higher plasma NfL levels exhibited more severe cognitive defects and brain hypometabolism, along with smaller hippocampal volume. These findings were validated using data from 619 participants in the Chinese Preclinical Alzheimer's Disease Study (C-PAS) cohort and 86 participants from a publicly available dataset. In addition, we found that increase in plasma NfL levels were predictive of faster cognitive decline and a higher likelihood of AD progression in men compared to women. In conclusion, sex differences influence the relationship between plasma NfL levels and AD symptoms. Men exhibit greater cognitive and neuropathological defects with rising plasma NfL levels, underscoring the need for considering sex when using NfL as a biomarker for neuronal damage in AD.

Molecular Psychiatry; https://doi.org/10.1038/s41380-025-03149-z

INTRODUCTION

Tracking the levels of neurodegeneration in Alzheimer's disease (AD) is crucial for accurate staging and for evaluating the efficacy of emerging treatments [1]. Diagnostic tools like positron emission tomography (PET) and measurements of Amyloid-β (Aβ) and tau protein levels in cerebrospinal fluid (CSF) [2] are commonly employed. Yet due to their invasiveness and high expense, current methods for assessing AD-related neurodegeneration still rely heavily on subjective cognitive assessments via various cognitive questionnaires. Recent development in plasma biomarkers aims to address this gap to provide objective, quantitative, and non-invasive markers that accurately reflect neurodegeneration and functional defects in AD patients [3–11].

Plasma neurofilament light (NfL) chain has emerged as a promising biomarker for AD. Elevated NfL levels in peripheral blood have been detected in both the early (prodromal) stages [12, 13] and advanced dementia stages [14] of sporadic AD patients, as well as in individuals with autosomal dominant AD [15, 16]. Increased plasma NfL levels have shown strong correlations with poorer cognitive performance, both cross-sectionally and longitudinally [12, 17, 18]. Furthermore, higher plasma NfL concentrations are associated with reduced whole brain and hippocampal volumes [19], increased severity of leukoencephalopathy [20], and metabolic decline in amyloid-positive individuals with AD [21]. Longitudinal studies also suggest that rapid increases in plasma NfL levels are linked to accelerated neuronal injury, brain atrophy, metabolic decline, and worsening cognitive function [14]. These evidences suggest that elevated plasma NfL reflects the degree of neurodegeneration in AD and could potentially be used to evaluate the effects of disease-modifying therapies. For example, plasma NfL concentration was included as a secondary outcome in clinical trials of Lecanemab, a recently FDA-approved treatment for AD [22].

Despite its potential, the implementation of plasma NfL in routine clinical practice faces challenges, particularly due to the lack of standardized measurement protocols [23]. Experts are now advocating for an international collaboration to define unified plasma NfL reference datasets that represent diverse populations around the world [23]. Key factors, such as sex, age, race and health status, need to be considered when defining these standards. Clarifying these blurs is essential for defining normal

[1]Department of Neurology, Zhongshan Hospital, Fudan University, Shanghai, China. [2]Department of Rehabilitation Medicine, Huashan Hospital, State Key Laboratory of Medical Neurobiology, Institute for Translational Brain Research, MOE Frontiers Center for Brain Science, MOE Innovative Center for New Drug Development of Immune Inflammatory Diseases, Fudan University, Shanghai, China. [3]Department of Nuclear Medicine & PET Center, Huashan Hospital, Fudan University, Shanghai, China. [4]Department of Neurology, Zhongnan Hospital, Wuhan University, Wuhan, China. [5]Department of Gerontology, Shanghai Jiaotong University Affiliated Sixth People's Hospital, Shanghai, China. [6]These authors contributed equally: Xiaoqin Cheng, Zhenghong Wang, Kun He, Yingfeng Xia. ✉email: fangxie@fudan.edu.cn; pyuan@fudan.edu.cn

Received: 15 March 2024 Revised: 22 May 2025 Accepted: 4 August 2025
Published online: 12 August 2025

plasma NfL ranges and may even necessitate the establishment of cut-off values for use in AD diagnostics.

While sex differences in AD have been studied extensively [24–30], there is limited research examining the role of sex in plasma NfL, and existing findings are mixed [14, 31–33]. Several studies reported no sex differences in plasma NfL levels in sporadic AD [14, 31, 33], while others indicated that female carriers of the Presenilin-1 E280A mutation experience greater NfL increases with age compared to male carriers [32]. These results contrast with findings from CSF studies, where men generally exhibit higher NfL concentrations than women [34, 35]. Studies on the interaction between sex and plasma NfL in relation to clinical manifestation of AD remain scarce. Recent study found the interaction effect of sex by plasma NfL levels could not predict cognitive decline in autosomal dominant AD mutation carriers [32]. To our knowledge, no study has systematically investigated the interaction effect of sex by plasma NfL on clinical manifestation in sporadic AD.

In the present study, we investigate sex difference in the relationship between plasma NfL concentration and cognition, neuropathological biomarkers, and clinical progression. Using data from the Alzheimer's Disease Neuroimaging Initiative (ADNI), We first explored sex-specific effects across cognitively unimpaired individuals, those with mild cognitive impairment (MCI), and AD patients. Upon identifying sex-specific trends in the ADNI cohort, we then sought to validate these findings using our own Chinese Preclinical Alzheimer's Disease Study (C-PAS) dataset as well as another publicly available dataset [19].

METHODS

Study design and participants

The data analyzed in this study included public data obtained from the ADNI and validation data obtained from our own cohort—C-PAS [36] and in a third open dataset from a previous study [19]. Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (*adni.loni.usc.edu*). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). The study was approved by the Institutional Review Board of each participating research site and all study subjects provided written informed consent. We first accessed data from ADNI cohort between Sep 7, 2005, and Sep 4, 2022. Participants with varying degrees of cognitive performance were deemed eligible if they had measurements of NfL concentrations and underwent [^{18}F] Florbetapir PET scans to assess brain Aβ deposition. A subset from the ADNI study had baseline and follow-up NfL, cognitive data, CSF biomarkers, and available imaging metrics (Supplementary Methods).

A cross-sectional dataset from C-PAS cohort, between Apr 2019 and Mar 2022, was collected for validation study. The study was approved by the Independent Ethics Committee "Huashan Hospital Institutional Review Board (HIRB), Fudan University" (approval number: KY-2017-406), and all participants gave written informed consent. Individuals in the C-PAS cohort with measurements of plasma NfL concentrations and underwent [^{18}F] Florbetapir PET scans were included.

The third dataset was obtained from openly shared dataset in Khalil et al. 2020, including 86 subjects with longitudinal plasma NfL concentration changes and brain volume changes [19].

Cognitive performance

Most subjects of ADNI underwent a series of cognitive performance tests, including the Mini-Mental State Examination (MMSE), the Montreal Cognitive Assessment (MoCA), the Clinical Dementia Rating–Sum of Box (CDR-SB), the Alzheimer's Diseases Assessment Scale-Cognitive Scales (ADAS-cog) (including ADAS-cog 11, ADAS-cog 13, and ADASQ4), and the Rey-Auditory-Verbal-Learning-Test (RAVLT) scales (including RAVLT_immediate, RAVLT_learning, RAVLT_forgetting, and RAVLT_perc_forgetting). In addition to the individual tests, we also evaluated composite scores for different cognitive domains, including memory, executive function, language, and visuospatial function derived from the ADNI neuropsychological test battery [37–39]. In C-PAS study, participants received MMSE and Montreal Cognitive Assessment Basic (MoCA-B), a useful tool for evaluating cognitive function in elderly individuals in China, and can effectively differentiate between those with normal cognition, MCI, and those with mild to moderate Alzheimer's disease, regardless of their education levels [40].

Plasma protein measurements

In ADNI, the blood sampling process adhered to protocol described before [12]. NfL, phosphorylated tau 181 (p-tau181) and glial fibrillary acidic protein (GFAP) were all determined using the ultrasensitive Single-Molecule-Array (SIMOA) technology platform.

For NfL detection, a blend of monoclonal antibodies, along with purified bovine NfL as a calibrator, was utilized in the assay. All plasma samples were measured in duplicate. The concentrations of all samples were above the detection limit, and the analytical sensitivity was <1 pg/mL [12]. The plasma collection process and determination method of plasma NfL concentrations in participants of C-PAS study were described before [36]. Briefly, the NF-light Assay Kit was used to measure the concentrations of plasma NfL. Plasma samples were diluted at a ratio of (1:4). Laboratory technicians, who were blinded to the clinical data, determined the plasma NfL levels by using Quanterix SIMOA HD-1 and Analyzer software [36].

For p-tau181 detection, two monoclonal antibodies (Tau12 and AT270) were used and N-terminal to middomain forms of p-tau181 were measured as described before [41].

GFAP was detected via a two-step digital immunoassay, which performed by Amsterdam UMC, VUmc, laboratory of Charlotte Teunissen Assay by using Simoa Neuro 4-plex E kit.

CSF Aβ and tau

The CSF samples were collected via lumbar puncture from participants in ADNI study, and analyzed using fully automated Elecsys immunoassays (Roche Diagnostics) to determine concentrations of CSF $A\beta_{1-42}$, total tau (t-tau), and p-tau181, as previously described [42].

Brain imaging data acquisition and processing

Structural MRI imaging data were retrieved from the LONI database (ida.loni.usc.edu). The procedures for ADNI brain imaging acquisition and processing have already been elaborated in detail at the website http://adni.loni.usc.edu/methods/documents. To put it simply, structural MRI scans are carried out on 3 T scanners by employing either a 3D MPRAGE or IR-SPGR T1-weighted sequence with sagittal slices and the spatial resolution of these scans is 1 mm^3. Subsequently, the scans are skull-stripped, segmented and parcellated with the help of FreeSurfer (version 5.1).

The [^{18}F] Florbetapir PET data of the ADNI participants were analyzed by Susan Landau and colleagues. The Cortical summary Florbetapir standard uptake value ratio (SUMMARYSUVR), which is intensity-normalized by the mean of whole cerebellum retention, was used to determine Aβ deposition, with SUMMARYSUVR values greater than or equal to 1.11 considered Aβ positive [43]. Fluorodeoxyglucose-PET (FDG-PET) data of the ADNI participants were analyzed by Chen K. and colleagues. FDG-PET SUVR, a volume-weighted average of a pons/vermis intensity-normalized region encompassing bilateral angular, inferior temporal gyrus, and posterior cingulate, was utilized to assess brain glucose metabolism as described previously [44].

Subjects in C-PAS study underwent structural MRI and [^{18}F] Florbetapir PET scans. The chief scanning parameters were described previously [36]. The volume of the hippocampus was measured by using the computational software Anatomy Toolbox (CAT12), which was implemented in Statistical Parametric Mapping (SPM12) software package. The visual evaluation method, based on the basic guidelines outlined by SNMMI [45] and the International Nuclear Medicine Consensus on the Clinical Use of Amyloid Positron Emission Tomography in Alzheimer's Disease [46], was used to determine whether the [^{18}F] Florbetapir PET was positive or negative.

Statistical analyses

The primary analytical approach employed in the study was a multi-level linear model. Across all cohorts, age, ApoE genotype, body-mass index

(BMI) and education were included as control variables whenever possible. In the ADNI cohort, the main effect was sex*NfL, with a random intercept assigned to each subject due to the longitudinal nature of the dataset. For each outcome studied, only the timepoints with NfL measurements were included for the analysis. In the C-PAS cohort, the main effect was also sex*NfL. However, due to the cross-sectional nature of this dataset, no random effects were incorporated.

To examine whether men and women differ in the relationship between longitudinal changes of plasma NfL and speed of cognitive decline, we first fitted linear trend lines between time and plasma NfL as well as time and aggregated cognitive scores respectively, based on longitudinal multiple measurements of each subject. The slope of these trend lines indicated the speed of longitudinal changes. We then fitted a linear regression model that controlled for covariates including age, ApoE genotype, BMI and education.

Regarding our third dataset, neither ApoE genotype nor education data were available. Since this publicly available database provided only the NFL change value rather than specific NFL values, we selected the NFL change value for analysis. The main effect here was sex*NfL change, without random effect.

To visualize the fitted model, we used the individual conditioned expectation plots [47]. For statistical comparisons, we examined whether the coefficients of the sex:NfL variable in the fitted models were significantly different from 0. All calculations were carried out with MATLAB 2021b software, and some plots were generated with GRAPHPAD Prism (Supplementary Methods).

In order to search for features correlated with the increase of plasma NfL levels, we constructed a partial least square regression (PLS) model [48]. Plasma NfL levels were used as response variables, with structural brain magnetic resonance imaging data, cognitive scores, cerebrospinal fluid biomarkers and glucose metabolism positron emission tomography data as predict variable. We included 10 PLS variables in the output, which explained about 80% variance. Projection calculations were carried out using 3–10 output variables. For visualization, only the first 3 variables were plotted (Supplementary Methods).

In order to examine the effect of plasma NfL to the conversion probability from non-AD to AD, we used a Cox regression model. This model is commonly used in the context of survival analysis. Our analysis can be interpreted as replacing the time in survival analysis with plasma NfL. We could control for the effect of other confounders in the Cox model for calculating sex dependent effect of plasma NfL levels. To visualize the model in an intuitive stair-plot manner, we omitted the effect of these confounders (Supplementary Methods).

RESULTS

Demographic characteristics

The demographic characteristics of participants from ADNI (Fig. S1) and from C-PAS (Fig. S2) were summarized in Tables 1, 2. In ADNI, 860 subjects met the inclusion criteria (Fig. S1). Among them, 412 (47.9%) were women and 448 (52.1%) were men. The mean (SD) age of all participants at baseline was 72.4 (7.2), and women (71.4 years) were younger than men (73.3 years). Women had an average education year of 15.7, and men had an average of 16.8. The ApoE-ε4 carrier rate was 44.7% in women and 45.5% in men. The median (IQR) plasma NfL concentration was 33.6 (19.9) pg/mL in women and 33.5 (21.1) pg/mL in men (Fig. S3). The baseline global Florbetapir PET SUVR had no difference between men and women ($p = 0.090$), while men had lower Composite Fluorodeoxyglucose-PET SUVR ($p = 0.010$). Women showed higher baseline MMSE ($p = 0.014$) and MoCA ($p = 0.001$) scores than men, while their CDR-SB, ADAS-Cog 11, and ADAS-Cog 13 scores were lower ($p = 0.004$ for CDR-SB, and $p < 0.001$ for both ADAS-Cog 11 and ADAS-Cog 13). The brain volume (Hippocampus, Whole brain, Entorhinal cortex, Ventricular) at baseline based on MRI showed that women had smaller brains than men (<0.001). Higher levels of CSF Aβ were found in women compared to men ($p = 0.015$).

The C-PAS study included a total of 619 participants (Fig. S2), of whom 388 (62.7%) were women and 231 (37.3%) were men (Table 2). Consistent with the ADNI data, women (63.7 years) were younger than men (66.2 years) ($p < 0.001$), and women had fewer years of education ($p = 0.007$). A total of 164 individuals (26.5%) were carriers of ApoE-ε4 gene, and there was no significant difference in the ApoE-ε4 carriers' rates between sexes ($p = 0.821$), however, the percentage of ApoE-ε4 carriers was quite lower in C-PSA study than in the ADNI study. Additionally, 459 individuals (74.2%) were found to be Aβ positive, with no significant differences existed between sexes ($p = 0.664$). The mean volume of the right hippocampal gray matter was greater in men than in women ($p = 0.012$). No significant difference was found in MMSE ($p = 0.685$) or MoCA-B ($p = 0.485$) between male and female subjects in the C-PSA study. Inconsistent with the ADNI data, a statistically significant difference was found in plasma NfL levels between sexes ($p = 0.025$), as the median (IQR) plasma NfL concentration was 13.7 (8.0) pg/mL in women and 15.4 (9.6) pg/mL in men. These comparisons help contextualize the demographic and biomarker differences, which are essential for further analyses, considering both sex and diagnostic differences in different races.

Sex differences in associations between plasma NfL levels and cognitive defects in AD

We initially delved into the data from the ADNI cohort to examine whether sex has an impact on the associations between the severity of cognitive defects and plasma NfL levels. By controlling for the effect of age, education, ApoE-ε4 status, BMI, and individual variability, we fitted a linear mixed effect (LME) model for the cognitive scores considering sex, plasma NfL levels, follow-up months, and their interactions. As the ADNI dataset reported multiple measurements for each subject over several years, we assigned a random intercept to each subject to account for the covariance among data from the same subject. This approach enabled us to appropriately analyze how sex might modify the associations between plasma NfL levels and cognitive defects (Fig. S4, Table S1).

For the CDR-SB score, we observed a significant interaction effect of sex and plasma NfL levels. Plasma NfL levels were positively correlated with CDR-SB scores in both sexes, indicating that higher NfL levels were associated with more severe cognitive impairment. Notably, this positive correlation was more pronounced in men. For each unit increase in plasma NfL, men exhibited a more substantial exacerbation of cognitive defect ($b = 0.0096$, 95%CI = 0.0010–0.018, $p = 0.029$, Fig. 1A). Consistently, we found similar trend of men showing more severe deficits with the same amount of plasma NfL increase for the other two commonly used cognitive scales, the Montreal Cognitive Assessment (MoCA, $b = -0.015$, 95%CI = −0.032–0.0024, $p = 0.093$, Fig. 1B) and Mini-Mental State Examination (MMSE, $b = -0.0092$, 95%CI = −0.023–0.0051, $p = 0.21$, Fig. 1C). These results collectively suggest that sex plays a crucial role in modulating the relationship between plasma NfL levels and cognitive defects, with men appearing to be more vulnerable to the adverse effects of elevated NfL levels on cognitive function.

Considering the variability for assessing cognitive scores, we derived a combinatorial metric using the first principal component of these three scores, additionally with others available in the ADNI dataset: RAVLTs series (RAVLT Immediate (sum of 5 trials), RAVLT Learning (trial 5 - trial 1), RAVLT Percent Forgetting)) and ADAS series (ADAS-cog 11, ADAS-cog 13, ADASQ4). This metric showed good correlations with different scores and was positively correlated with cognitive defect (Fig. S4B). The same LME analysis as described above using this metric as the response variable again demonstrated significant sex-dependent difference ($b = 0.18$, 95%CI = 0.058–0.29, $p = 0.0034$, Fig. 1D). We further examined composite scores representing different cognitive domains available from ADNI, and found that functional domains related to language and memory showed consistent sex-dependent effects (Fig. S5). Another visualization for the correlation between plasma NfL and these cognitive scores in two sexes, presented as a partial dependence plot (Fig. S6), also supported

Table 1. Demographic characteristics of participants at baseline in ADNI study shown by sex.

Characteristic		Female ($n = 412$)	Male ($n = 448$)	Total ($n = 860$)	*P* value
Age at baseline, years (SD)		71.4 (7.1)	73.3 (7.2)	72.4 (7.2) [$n = 860$]	<0.001
Education (SD)		15.7 (2.6)	16.8 (2.6)	16.3 (2.6) [$n = 860$]	<0.001
Apoe4 carriers, No. (%)		184 (44.7%)	204 (45.5%)	388 (45.1%)	0.797
Aβ positive, No.(%)		237 (57.5%)	241 (53.8%)	478 (55.6%)	0.271
Diagnose	Cognitively normal, No. (%)	155 (37.6%)	128 (28.5%)	283 (32.9%)	0.016
	Mild cognitive impairment, No. (%)	199 (48.3%)	242 (54.0%)	441 (51.3%)	
	Dementia, No. (%)	58 (14.1%)	78 (17.4%)	136 (15.8%)	
Plasma NfL level, median (IQR), ng/L		33.6 (19.9)	33.5 (21.1)	33.6 (20.2)	0.642
PET biomarkers at baseline	Composite Fluorodeoxyglucose-PET, median (IQR), SUVR	1.28 (0.10) [$n = 412$]	1.27 (0.09) [$n = 446$]	1.27 (0.10) [$n = 858$]	0.010
	Global Aβ PET, median (IQR), SUVR	1.16 (0.37)	1.13 (0.38)	1.14 (0.37)	0.090
Cognitive score at baseline	MMSE, median (IQR)	29.0 (4.0)	28.0 (3.0)	29.0 (4.0)	0.014
	MOCA, median (IQR)	24.0 (6.0) [$n = 409$]	23.0 (5.0) [$n = 441$]	24.0 (5.0) [$n = 850$]	0.001
	CDR-SB, median (IQR)	1.0 (2.0)	1.0 (2.5)	1.0 (2.0)	0.004
	ADAS-Cog 11, median (IQR)	7.0 (7.0) [$n = 412$]	9.0 (8.0) [$n = 446$]	8.0 (7.3) [$n = 858$]	<0.001
	ADAS-Cog13, median (IQR)	11.0 (12.0) [$n = 410$]	14.0 (11.0) [$n = 446$]	13.0 (12.0) [$n = 856$]	<0.001
MRI Imaging measure at baseline	Hippocampus, mean (SD), mm3	6894.8 (1085.8) [$n = 376$]	7171.3 (1171.3) [$n = 401$]	7037.5 (1138.4) [$n = 777$]	0.001
	Whole rain, mean (SD), mm3	995866.8 (84742.0) [$n = 395$]	1098384.5(99292.9) [$n = 432$]	1049419.0(105807.3) [$n = 827$]	<0.001
	Entorhinal cortex, mean (SD), mm3	3424.7 (658.7) [$n = 367$]	3790.9 (742.7) [$n = 381$]	3611.2 (725.7) [$n = 748$]	<0.001
	Ventricular volume, mean (SD), mm3	31093.4 (18143.3) [$n = 384$]	43065.1 (21640.3) [$n = 416$]	37318.7 (20900.8) [$n = 800$]	<0.001
CSF biomarkers at baseline	Aβ level, median (IQR), ng/L	843.8 (519.0) [$n = 294$]	772.4 (524.0) [$n = 342$]	805.7 (517.0) [$n = 636$]	0.015
	p-tau level, median (IQR), ng/L	23.2 (17.2) [$n = 370$]	22.7 (14.3) [$n = 411$]	23.0 (15.8) [$n = 781$]	0.315

There were 412 (47.9%) women and 448 (52.1%) men in our sample of 860 participants. Across all participants, 283(32.9%) were cognitively normal (CN), 441 (51.3%) were diagnosed with MCI, 136(15.8%) were diagnosed with dementia, and 478(55.6%) were Aβ positive. The mean age was 72.4 years old and the average years of education was 16.3. Women in our sample were younger and had fewer years of education.

Table 2. Demographic characteristics of participants in C-PAS study shown by sex.

Characteristic		Female ($n = 388$)	Male ($n = 231$)	Total ($n = 619$)	*P* value
Age at baseline, years (SD)		63.7 (7.5) [$n = 388$]	66.2 (7.0) [$n = 231$]	64.6 (7.4) [$n = 619$]	<0.001
Education (SD)		11.2 (3.6) [$n = 388$]	12.0 (3.4) [$n = 231$]	11.5 (3.6) [$n = 619$]	0.007
Apoe4 carriers, No. (%)		104 (26.8)	60 (26.0)	164 (26.5)	0.821
Aβ positive, No. (%)		290 (74.7%)	169 (73.2%)	459 (74.2%)	0.664
Gray matter Aβ PET, median (IQR), SUVR		1.25 (0.13) [$n = 386$]	1.23 (0.14) [$n = 231$]	1.24 (0.14) [$n = 617$]	0.007
Diagnose	Cognitively normal, No. (%)	237 (61.1)	137 (59.3)	374 (60.4)	0.685
	Mild cognitive impairment, No. (%)	94 (24.2)	54 (23.4)	148 (23.9)	
	Dementia, No. (%)	57 (14.7)	40 (17.3)	97 (15.7)	
Plasma NfL level, median (IQR), pg/mL		13.7 (8.0)	15.4 (9.6)	14.4 (8.6)	0.025
Cognitive score at baseline	MMSE, median (IQR)	27.0 (4.0)	27.0 (4.0)	27.0 (4.0)	0.685
	MoCA-B, median (IQR)	24.0 (7.0)	24.0 (6.0)	24.0 (7.0)	0.485
MRI Imaging measure	Left Hippocampus gray matter volume, mean (SD), mm^3	2.5 (0.3) [$n = 388$]	2.5 (0.4) [$n = 231$]	2.5 (0.3) [$n = 619$]	0.168
	Right Hippocampus gray matter volume, mean (SD), mm^3	2.8 (0.4) [$n = 388$]	2.9 (0.4) [$n = 231$]	2.9 (0.4) [$n = 619$]	0.012

There were 388 (62.7%) women and 231 (37.3%) men in our sample of 619 participants. Across all participants, 374(60.4%) were cognitively normal (CN), 148 (23.9%) were diagnosed with MCI, 97(15.7%) were diagnosed with dementia, and 459(74.2%) were Aβ positive. The mean age was 64.6 years old and the average years of education was 11.5. Women in our sample were younger and had fewer years of education. To account for sex differences in diagnostic status we additionally adjusted for the effect of diagnosis in subsequent analyses.

the positive relationship. Together, these data indicated that the same amount of plasma NfL increase is correlated with a more severe cognitive deficit in men than in women.

To test whether the observed sex difference between plasma NfL and cognitive defect represents a general rule for other biomarkers, we examined two additional plasma biomarkers that were previously reported to reflect cognitive decline: phosphorylated tau 181 [6] and glial fibrillary acidic protein [10]. We applied a similar linear regression method from the same ADNI cohort. The results show that while both biomarkers show good correlation with cognitive defect as reported, phosphorylated tau 181 showed the opposite sex difference from NfL, consistent with a previous report [30], and GFAP showed no sex difference (Fig. S7). These results suggest that the sex difference in the relationship between plasma NfL and cognition is unlikely due to unspecific sex difference in brain debris clearance or periphery metabolism.

Sex differences in associations between plasma NfL levels and severity of AD-related neuropathology

Given the above results regarding cognitive scores, we next examined whether sex also modified associations between plasma NfL levels and AD-related brain atrophy. To do this, we constructed linear regression models using T1-weighted structural MRI data from individual brain regions as the response variable. By color-coding each brain region with the t-values of the sex-plasma NfL coefficients in the model (Table S2), we visualized the sex-dependent effect. Our findings revealed that, in several brain regions, compared to women, men exhibited a more substantial reduction in cortical thickness and subcortical volume in relation to a unit increase in plasma NfL levels. Notably, this effect was particularly significant in the hippocampus (Fig. 2A–C, hippocampus $b = -3.0$, 95% $CI = -5.13 \sim -0.89$, $p = 0.0054$). In addition to T1-weighted MRI data, we also explored the sex-dependent effect on brain energy metabolism using FDG-PET data, and again found that men showed more severe brain hypometabolism with each unit increase of plasma NfL levels compared to women ($b = -5.5$, 95%$CI = -10 \sim -0.89$, $p = 0.019$, Fig. 2D).

Considering that multiple parameters related to AD progress showed a sex-dependent relationship to plasma NfL, we next attempted to extract a quantitative index encompassing all these variables as an overall measurement of AD manifestation. To this end, we performed a PLS using all available biomarkers including cognition scales, MRI and PET imaging data, and cerebrospinal fluid amyloid and tau proteins, with plasma NfL levels as the response variable. This allowed us to extract linear combinations of different biomarkers that closely reflect plasma NfL fluctuations. By color-coding each individual with their sex and clinical diagnosis, we found a clear direction for AD progression in the first three PLS dimensions (Fig. 2E). This allowed us to delineate the centroid from control subjects to that of the AD subjects. And we used the connection between the two centroid points as the direction for disease progression. Furthermore, we calculated the projections to this direction from each individual using the first 10 PLS dimensions (summed explained variance 78.7%, Fig. S8A, B), with incremental bins of plasma NfL levels for both sexes. We found that men showed a stronger linear correlation between NfL increase and projected AD progression ($b = 8.2$, 95%$CI = 5.2 \sim 11$, $r = 0.77$, $p < 0.0001$, Fig. 2F) than that of the women ($b = 4.8$, 95%$CI = -0.49$–10, $r = 0.27$, $p = 0.071$, Fig. 2F). This effect was consistent when the correlations were calculated through the first 3 to first 10 PLS dimensions, indicating a robust sex effect (Fig. S8C–E).

Sex differences in the plasma NfL levels-associated risks of converting to dementia

Having established the sex differences in the correlation of plasma NfL and cognitive as well as neuropathological defects, we next examined whether women and men differ in the relationship between longitudinal change of plasma NfL and speed of cognitive decline. For each subject with multiple measurements, we fitted linear trend lines between time and plasma NfL as well as time and aggregated cognitive scores, respectively. The slope of these trend lines indicated the speed of longitudinal changes. We then fitted a linear regression model that controlled for covariates including age, ApoE genotype, BMI, and education. We found a significant effect for the interaction between sex and slope of plasma NfL changes, showing more severe cognitive decline in men ($b = 0.17$, $SE = 0.082$, $p = 0.036$, Fig. 3A).

The above analysis indicated that the same increase of plasma NfL reflected more exacerbated cognitive decline in men than women. Thus, we postulated that the association between plasma

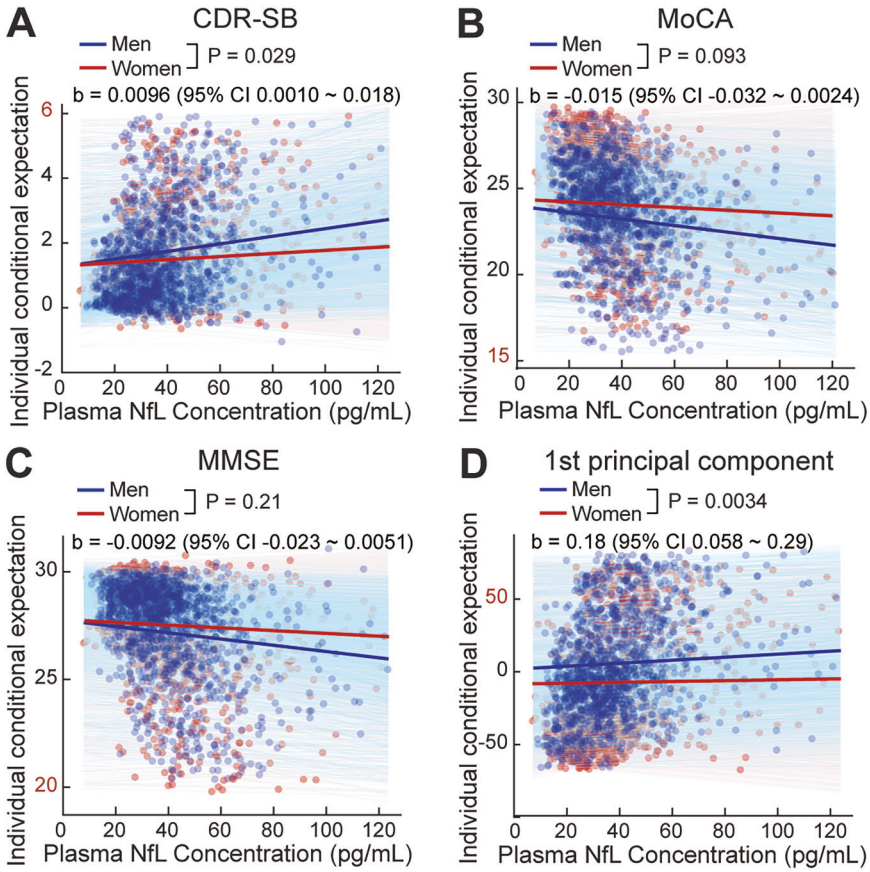

Fig. 1 Increased plasma NfL levels reflect more severe cognitive deficit in men. Individual conditional expectation plots demonstrate sex differences in the association between plasma NfL levels and worse performance in CDR **A**, MMSE **B**, MoCA **C** and 1 st principal component of cognition scales **D**, by using a linear mixed effect (LME) model (see text for details). The red mark on the vertical axis represents the direction of cognitive function deterioration. The cognition scales calculated in the 1 st principal component including CDR, MMSE, MoCA, AVLTs series (RAVLT Immediate (sum of 5 trials), RAVLT Learning (trial 5 - trial 1), RAVLT Forgetting (trial 5 - delayed), and RAVLT Percent Forgetting)) and ADAS series (ADAS-cog 11, ADAS-cog 13, ADASQ4). *P* values report the significance of the sex-plasma NfL interaction coefficients in the LME model.

NfL and clinical diagnosis to AD is also sex-dependent. To this end, we plotted a Kaplan-Meier survival curve using the plasma NfL levels, and depicted the probability of AD-free survival at each concentration ranges (Fig. 3B). While we observed a trend that men showed higher risk of converting to AD with unit increase of plasma NfL, the associated Cox proportional hazard model showed no statistical significance. We suspected this is due to relatively high amount of lost follow-up data in this cohort. To improve the statistical power, we applied a bootstrap resample method. We generated a distribution of the sex-NfL interaction coefficients in the Cox model, which showed significant difference from the shuffled control (mean = 0.33, 95%CI = 0.33–0.34, $p < 0.001$, Fig. 3C), supporting the notion of higher risk of converting to AD with the same amount of NfL increase in men. To examine the robustness of the sex effect, we tested different number of bootstrap sampling and found that mild up-sampling (>50) was capable of boosting statistical power (Fig. 3D). We further quantified the hazard ratios of men to women risks given the same amount of plasma NfL increase, and found that the sex difference grew monotonically with age (Fig. 3E), indicating that sex-dependent differences of plasma NfL and AD risk is more prominent in the elderly.

Sex differences in association between plasma NfL levels and cognitive defects in validation cohort

To examine whether the sex-dependent correlation between plasma NfL levels and AD manifestation existed outside the ADNI dataset, we further explored these correlations in our validation cohort from C-PAS study (Table S2). We constructed similar linear models as those used in the ADNI dataset. However, since this cohort was a cross-sectional dataset, we eliminated the follow-up months variable and the random intercepts from subjects, and applied a robust fitting parameter to ensure correct intercepts. Consistent with our results from ADNI cohort, we found significant interaction between sex and plasma NfL levels in correlation with MMSE (b = −0.059, 95%CI = −0.097 ~ −0.020, $p = 0.0027$, Fig. 4A), MoCA (b = −0.070, 95%CI = −0.13 – −0.013, $p = 0.017$, Fig. 4B), and hippocampus volume (b = −0.00096, 95%CI = −0.019 – −0.00041, $p = 0.040$, Fig. 4C). In all these measurements, men showed more prominent functional and structural defects associated with unit increase of plasma NfL, consistent with our findings in the ADNI cohort.

We further examined the effect of sex on the relationship between plasma NfL change with whole brain volume in a third dataset that is openly available from a previous study [19]. The model for this set did not include ApoE genotype or education due to the absence of the information. Nevertheless, we found a pronounced effect that plasma NfL increase was associated with more severely reduced brain volume in men (b = −0.018, 95% CI = −0.034 – −0.0017, $p = 0.031$, Fig. S9), consistent with our results from other cohorts.

DISCUSSION

Sex constitutes an important factor for AD patient stratification and personalized treatment. In this study, we performed an

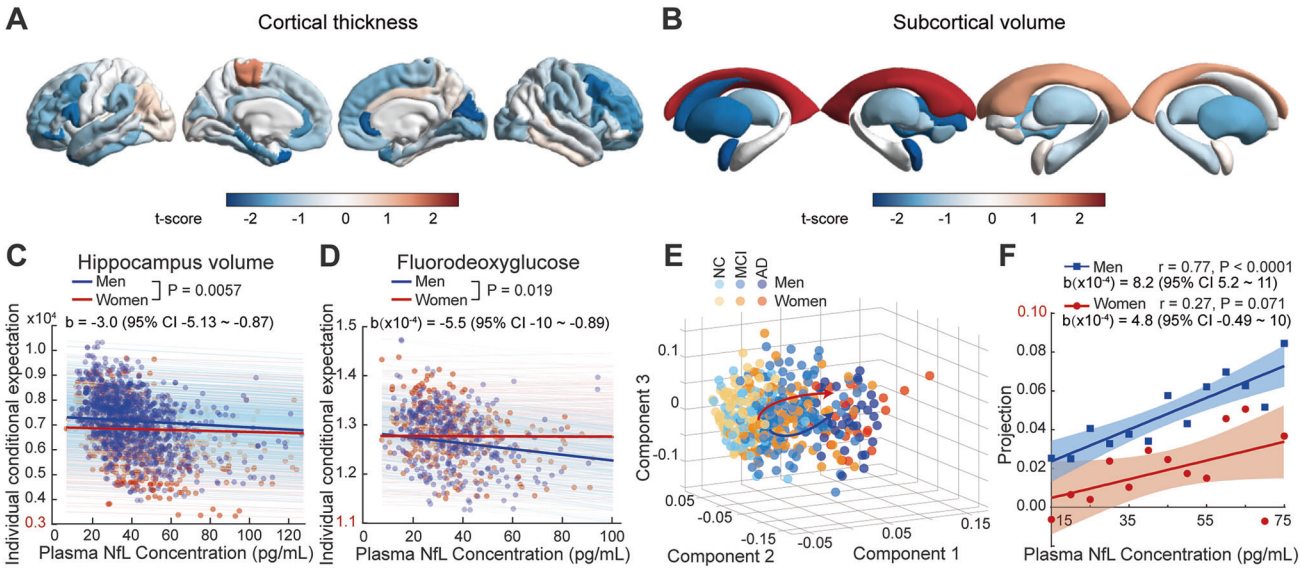

Fig. 2 Increased plasma NfL levels indicate more pronounced AD-related neuropathology in men. LME model was fitted for the T1-weighted structural MRI data. **A** and **B** visualize the t-score data on MRI segmentation structures obtained from the sex-plasma NfL interaction term in the LME regression and depict that sex modified the associations between plasma NfL levels and cortical thickness **A**, and subcortical volume **B**. **C, D**. Individual conditional expectation plots depicting that men showed more substantial reduction in hippocampus volume **C** and brain hypometabolism **D** with unit increase of plasma NfL levels than women. In **C** and **D**, *P* values report the significance of the sex-plasma NfL interaction coefficients in the LME model. **E**. A PLS regression was performed for a quantitative index encompassing all available biomarkers including cognition scales, MRI and PET imaging data, and cerebrospinal fluid amyloid or tau proteins, with plasma NfL levels as the response variable. Individual dot represents one measured timepoint from one person, with color coded for sex and clinical diagnosis. Arrows indicate directions for AD progression. **F**. Quantification of the PLS projection of the first 10 PLS dimensions to the direction for disease progression, with incremental bins of plasma NfL levels for both sexes. Men showed a stronger linear correlation between NfL increase and projected AD progression than that of women. *P* values and R values represent Pearson's correlation analysis.

in-depth investigation regarding whether sex modifies the associations between plasma NfL concentration and cognition as well as neuropathologic biomarkers as manifestation of dementia by using data from the ADNI study and our C-PAS cohort, which has not been done before to the best of our knowledge. While previous studies showed that increased plasma NfL levels were correlated with worsening of MMSE scores, CDR-SB and ADAS-cog scores, loss of hippocampal volume and cortical thickness, increases in the total and phosphorylated tau protein level, reduction in FDG-PET, and expansion of ventricular volume in both sexes [14], our study expands prior understanding and showed that the relative strength of the association between plasma NfL and these parameters varies by sex. Furthermore, we found that the sex-dependent relationship was consistent across different populations, as demonstrated in both the predominantly Caucasian ADNI cohort and our validation cohort of Asian participants.

We found that with the same amount of increase in the plasma NfL levels, men exhibit more severe defects related to AD compared to women. These results are surprising considering many reports have described that women are more impacted by Alzheimer's disease than men. Women are at significantly greater risk of developing the disease, and appear to suffer a greater cognitive deterioration than men at the same stage of the disease [25]. Notably, we did not observe the same sex difference for plasma phosphorylated tau 181 or GFAP (Fig. S7), suggesting a NfL-specific mechanism.

The mechanism underlying the sex differences we observed is not clear, but we propose several hypotheses as detailed below. First, sex-specific microglia-driven neuroinflammation may explain the observed pattern where each unit increase in plasma NfL levels results in more severe cognitive and neuropathological defects in men. Prior research showed sex differences in microglial activation [49] and demyelination [50], which are closely linked to NfL release [51]. In our previous study, clearing microglia with the colony-stimulating factor 1 receptor inhibitor PLX3397 in female Tg2541 mice extended lifespan and lowered plasma NfL levels. However, in male Tg2541 mice, the same treatment led to increased NfL levels and shortened lifespan [52]. These results suggest that microglia-related neuroinflammation could significantly affect plasma NfL production in Alzheimer's and other tau-related neurodegenerative diseases, with a pronounced sex-dependent effect. Second, sex differences in Blood-Brain Barrier (BBB) integrity may influence NfL release levels. Animal studies have demonstrated that female sex hormones provide protective effects on BBB permeability under both normal and pathological conditions [53, 54]. In a large human study involving over 20,000 participants, females exhibited significantly lower CSF/serum albumin ratios compared to males, suggesting a less permeable BBB in women [55]. Another human study using dynamic contrast-enhanced - MRI found that females showed better BBB integrity in the cingulate and occipital cortices compared to males [56]. Based on these findings, we hypothesize that men may experience more pronounced BBB dysfunction in AD, contributing to higher plasma NfL levels. Future research is required in dissecting the mechanism underlying the sex-dependent association between plasma NfL increase and AD manifestation.

Our findings could have important implications for the use of plasma NfL as a tool for tracking neurodegeneration in AD. Palermo et al. noted that plasma NfL holds significant potential for risk assessment, disease monitoring, and stratification of AD [57]. However, the clinical application of NfL as a biomarker requires the establishment of precise cutoffs to distinguish high-risk individuals from low-risk ones with optimal sensitivity. In our study, men exhibited more prominent cognitive defects and worse biomarker profiles at the same plasma NfL concentrations as women. Without sex-specific baselines, one sex may face biased diagnosis. Implementing sex-adjusted NfL reference values could enhance diagnostic accuracy. Moreover, monitoring dynamic changes in NfL levels is critical for tracking AD progression, as

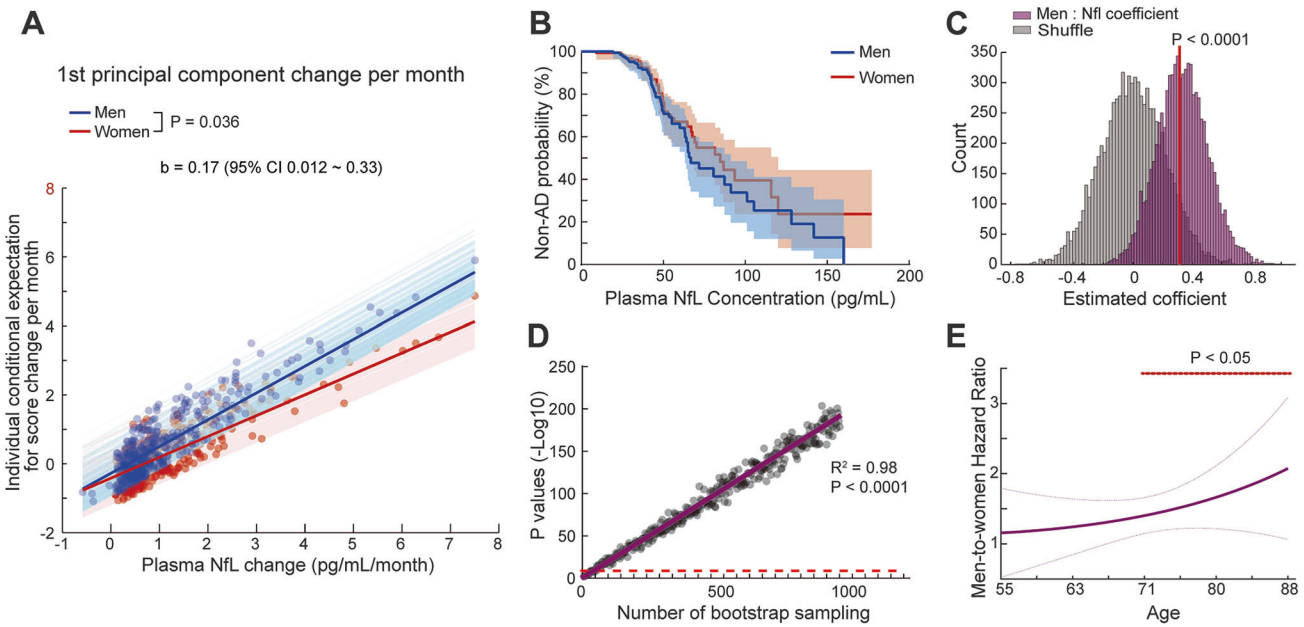

Fig. 3 Increased plasma NfL levels predict higher risks of converting to dementia in men. A. Individual conditional expectation plots demonstrate that increased plasma NfL levels reflect more severe cognitive decline in men by using a linear regression model (see text for details). The red mark on the vertical axis represents the direction of cognitive function deterioration. **B**. A Kaplan-Meier survival curve was plotted to depict the probability of AD-free survival at each plasma NfL concentration ranges in men and women. **C**. Distribution of the sex-NfL interaction coefficients in the Cox model using a bootstrap resample method. *P* value indicates t-test between the actual data distribution and shuffled control. **D**. *P* values between the actual data and shuffled control with different number of bootstrap samples. Purple line indicates a fitted linear regression line. Red dash line shows the threshold at $p = 0.05$. **E**. The hazard ratios of men to women risks given the same amount of plasma NfL increase at each age group. Dotted lines indicated 95% confidence interval.

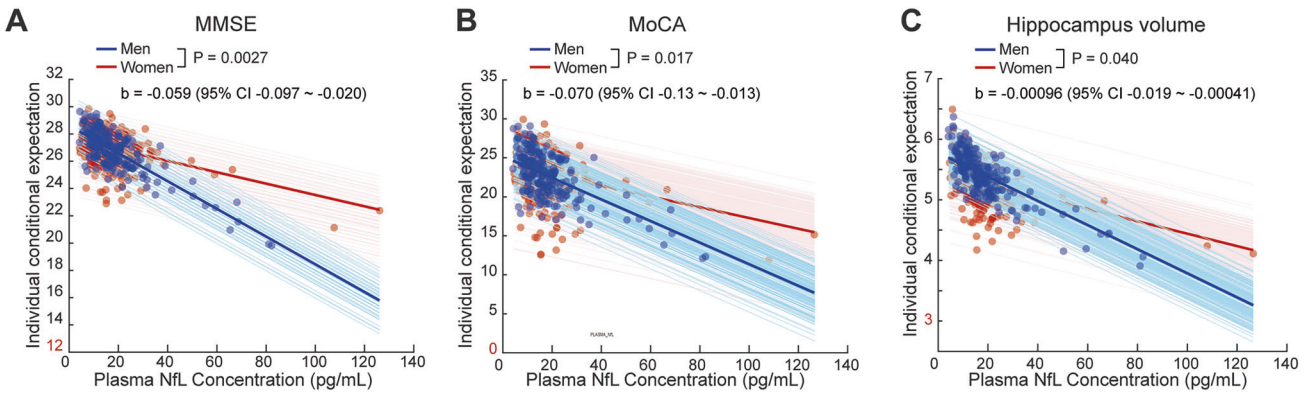

Fig. 4 Increased plasma NfL levels reflect more severe cognitive deficit and more substantial reduction in hippocampus volume in men in the C-PAS cohort. Individual conditional expectation plots showing significant interactions between sex and plasma NfL levels in correlation with MMSE **A**, MoCA-B **B**, and hippocampus volume **C**. Men showed more prominent functional and structural deterioration associated with unit increase of plasma NfL. *P* values report the significance of the sex-plasma NfL interaction coefficients in the linear regression model.

men and women may experience distinct patterns of NfL elevation over the course of the disease. Future longitudinal studies should focus on sex-specific NfL trajectories to refine disease progression models. Besides, sex differences in neurodegeneration and NfL release may also influence treatment responses. For instance, therapies targeting neuroinflammation may need to account for NfL dynamics based on sex. Tailoring treatment monitoring to sex-specific NfL responses could enhance personalized approaches in AD care. In conclusion, future research should explore whether sex-specific plasma NfL cutoffs improve the sensitivity of NfL as a predictive biomarker for AD and as a screening tool for clinical trials.

The present study has several limitations. Firstly, the cognitive functions were evaluated via various scoring systems with relatively high subjective variances. We tried to reduce the measurement error by deriving an aggregated cognitive score with principal component analysis, yet objective measurements such as functional magnetic resonance imaging or electrophysiological features could be used in future studies to further validate our findings. Secondly, the current sample size was limited, particularly for the survival analysis. While we believe that our bootstrap method is statistically sound, we hope to validate this conclusion with a larger dataset in the future. Thirdly, it remains to be seen whether the sex difference we observed remains consistent in patients receiving disease modifying treatments including passive immunizations that are currently available. The use of plasma NfL as a biomarker for AD-related neurodegeneration under such circumstance requires further investigation.

In conclusion, our research showed a sex difference in the association of plasma NfL and AD manifestation. Specifically, while

increased plasma NfL levels correlated with AD-related defects in both sexes, men showed more severe deficits with every unit increase of plasma NfL compared to women, as measured by cognition scales, brain volume and hypometabolism, and clinical diagnosis. This effect was consistent in two additional cohorts studied in this work. While the mechanism underlying this sex difference is unknown, our work indicated that sex may impact the clinical interpretation of plasma NfL concentrations and should be considered as a potential modifier of the prognostic utility of plasma NfL in future studies.

DATA AVAILABILITY

ADNI data are publicly available at: https://adni.loni.usc.edu. Data from C-PAS study will be shared by request from a qualified academic investigator for the sole purpose of replicating procedures and results presented in the article. The third longitudinal open dataset can be download at: https://www.nature.com/articles/s41467-020-14612-6#Sec16. Analysis code used in this study is available upon reasonable request.

REFERENCES

1. Therriault J, Schindler SE, Salvadó G, Pascoal TA, Benedet AL, Ashton NJ, et al. Biomarker-based staging of Alzheimer disease: rationale and clinical applications. Nat Rev Neurol. 2024;20:232–44.
2. Rauchmann BS, Schneider-Axmann T, Perneczky R. Alzheimer's disease neuroimaging I. associations of longitudinal plasma p-tau181 and NfL with tau-PET, Abeta-PET and cognition. J Neurol Neurosurg Psychiatry. 2021;92:1289–95.
3. Jiang Y, Uhm H, Ip FC, Ouyang L, Lo RMN, Cheng EYL, et al. A blood-based multi-pathway biomarker assay for early detection and staging of Alzheimer's disease across ethnic groups. Alzheimers Dement. 2024;20:2000–15.
4. Milà-Alomà M, Ashton NJ, Shekari M, Salvadó G, Ortiz-Romero P, Montoliu-Gaya L, et al. Plasma p-tau231 and p-tau217 as state markers of amyloid-β pathology in preclinical Alzheimer's disease. Nat Med. 2022;28:1797–801.
5. Mattsson-Carlgren N, Salvadó G, Ashton NJ, Tideman P, Stomrud E, Zetterberg H, et al. Prediction of longitudinal cognitive decline in preclinical Alzheimer disease using plasma biomarkers. JAMA Neurol. 2023;80:360–9.
6. Jiang Y, Zhou X, Ip FC, Chan P, Chen Y, Lai NCH, et al. Large-scale plasma proteomic profiling identifies a high-performance biomarker panel for Alzheimer's disease screening and staging. Alzheimers Dement. 2022;18:88–102.
7. Chatterjee P, Pedrini S, Doecke JD, Thota R, Villemagne VL, Doré V, et al. Plasma Aβ42/40 ratio, p-tau181, GFAP, and NfL across the Alzheimer's disease continuum: a cross-sectional and longitudinal study in the AIBL cohort. Alzheimers Dement. 2023;19:1117–34.
8. Shen XN, Huang SY, Cui M, Zhao QH, Guo Y, Huang YY, et al. Plasma glial fibrillary acidic protein in the Alzheimer disease continuum: relationship to other biomarkers, differential diagnosis, and prediction of clinical progression. Clin Chem. 2023;69:411–21.
9. Janelidze S, Barthélemy NR, Salvadó G, Schindler SE, Palmqvist S, Mattsson-Carlgren N, et al. Plasma phosphorylated Tau 217 and Aβ42/40 to predict early brain Aβ accumulation in people without cognitive impairment. JAMA Neurol. 2024;81:947–57.
10. Guo Y, You J, Zhang Y, Liu WS, Huang YY, Zhang YR, et al. Plasma proteomic profiles predict future dementia in healthy adults. Nat Aging. 2024;4:247–60.
11. Ashton NJ, Brum WS, Di Molfetta G, Benedet AL, Arslan B, Jonaitis E, et al. Diagnostic accuracy of a plasma phosphorylated tau 217 immunoassay for Alzheimer disease pathology. JAMA Neurol. 2024;81:255–63.
12. Mattsson N, Andreasson U, Zetterberg H, Blennow K. Alzheimer's disease neuroimaging I. association of plasma neurofilament light with neurodegeneration in patients with Alzheimer disease. JAMA Neurol. 2017;74:557–66.
13. de Wolf F, Ghanbari M, Licher S, McRae-McKee K, Gras L, Weverling GJ, et al. Plasma tau, neurofilament light chain and amyloid-beta levels and risk of dementia; a population-based cohort study. Brain. 2020;143:1220–32.
14. Mattsson N, Cullen NC, Andreasson U, Zetterberg H, Blennow K. Association between longitudinal plasma neurofilament light and neurodegeneration in patients with Alzheimer disease. JAMA Neurol. 2019;76:791–9.
15. Preische O, Schultz SA, Apel A, Kuhle J, Kaeser SA, Barro C, et al. Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. Nat Med. 2019;25:277–83.
16. Quiroz YT, Zetterberg H, Reiman EM, Chen Y, Su Y, Fox-Fuller JT, et al. Plasma neurofilament light chain in the presenilin 1 E280A autosomal dominant Alzheimer's disease kindred: a cross-sectional and longitudinal cohort study. Lancet Neurol. 2020;19:513–21.
17. Moscoso A, Grothe MJ, Ashton NJ, Karikari TK, Lantero Rodríguez J, Snellman A, et al. Longitudinal associations of blood phosphorylated tau181 and neurofilament light chain with neurodegeneration in Alzheimer disease. JAMA Neurol. 2021;78:396–406.
18. He L, Morley JE, Aggarwal G, Nguyen AD, Vellas B, de Souto Barreto P, et al. Plasma neurofilament light chain is associated with cognitive decline in non-dementia older adults. Sci Rep. 2021;11:13394.
19. Khalil M, Pirpamer L, Hofer E, Voortman MM, Barro C, Leppert D, et al. Serum neurofilament light levels in normal aging and their association with morphologic brain changes. Nat Commun. 2020;11:812.
20. Dittrich A, Ashton NJ, Zetterberg H, Blennow K, Simrén J, Geiger F, et al. Plasma and CSF NfL are differentially associated with biomarker evidence of neurodegeneration in a community-based sample of 70-year-olds. Alzheimers Dement. 2022;14:e12295.
21. Benedet AL, Ashton NJ, Pascoal TA, Leuzy A, Mathotaarachchi S, Kang MS, et al. Plasma neurofilament light associates with Alzheimer's disease metabolic decline in amyloid-positive individuals. Alzheimers Dement. 2019;11:679–89.
22. van Dyck CH, Swanson CJ, Aisen P, Bateman RJ, Chen C, Gee M, et al. Lecanemab in early Alzheimer's disease. N Engl J Med. 2023;388:9–21.
23. Abdelhak A, Kuhle J, Green AJ. Challenges and opportunities for the promising biomarker blood neurofilament light chain. JAMA Neurol. 2023;80:542–3.
24. 2023 Alzheimer's disease facts and figures. Alzheimers Dement. 2023;19:1598–695.
25. Laws KR, Irvine K, Gale TM. Sex differences in Alzheimer's disease. Curr Opin Psychiatry. 2018;31:133–9.
26. Liesinger AM, Graff-Radford NR, Duara R, Carter RE, Hanna Al-Shaikh FS, Koga S, et al. Sex and age interact to determine clinicopathologic differences in Alzheimer's disease. Acta neuropathol. 2018;136:873–85.
27. Filon JR, Intorcia AJ, Sue LI, Vazquez Arreola E, Wilson J, Davis KJ, et al. Gender differences in Alzheimer disease: brain atrophy, histopathology burden, and cognition. J Neuropathol Exp Neurol. 2016;75:748–54.
28. Oveisgharan S, Arvanitakis Z, Yu L, Farfel J, Schneider JA, Bennett DA. Sex differences in Alzheimer's disease and common neuropathologies of aging. Acta neuropathol. 2018;136:887–900.
29. Babapour Mofrad R, Tijms BM, Scheltens P, Barkhof F, van der Flier WM, Sikkes SAM, et al. Sex differences in CSF biomarkers vary by Alzheimer disease stage and APOE ε4 genotype. Neurology. 2020;95:e2378–e2388.
30. Tsiknia AA, Edland SD, Sundermann EE, Reas ET, Brewer JB, Galasko D, et al. Sex differences in plasma p-tau181 associations with Alzheimer's disease biomarkers, cognitive decline, and clinical progression. Mol Psychiatry. 2022;27:4314–22.
31. Baldacci F, Lista S, Manca ML, Chiesa PA, Cavedo E, Lemercier P, et al. Age and sex impact plasma NFL and t-Tau trajectories in individuals with subjective memory complaints: a 3-year follow-up study. Alzheimers Res Ther. 2020;12:147.
32. Vila-Castelar C, Chen Y, Langella S, Lopera F, Zetterberg H, Hansson O, et al. Sex differences in blood biomarkers and cognitive performance in individuals with autosomal dominant Alzheimer's disease. Alzheimers Dement. 2023;19:4127–38.
33. Brickman AM, Manly JJ, Honig LS, Sanchez D, Reyes-Dumeyer D, Lantigua RA, et al. Plasma p-tau181, p-tau217, and other blood-based Alzheimer's disease biomarkers in a multi-ethnic, community study. Alzheimers Dement. 2021;17:1353–64.
34. Skillbäck T, Blennow K, Zetterberg H, Shams S, Machado A, Pereira J, et al. Sex differences in CSF biomarkers for neurodegeneration and blood-brain barrier integrity. Alzheimers Dement. 2021;13:e12141.
35. Mielke MM, Syrjanen JA, Blennow K, Zetterberg H, Skoog I, Vemuri P, et al. Comparison of variables associated with cerebrospinal fluid neurofilament, total-tau, and neurogranin. Alzheimers Dement. 2019;15:1437–47.
36. Cui L, Huang L, Pan FF, Wang Y, Huang Q, Guan YH, et al. Chinese preclinical Alzheimer's disease study (C-PAS): design and challenge from PET acceptance. J Prev Alzheimers Dis. 2023;10:571–80.
37. Gibbons LE, Carle AC, Mackin RS, Harvey D, Mukherjee S, Insel P, et al. A composite score for executive functioning, validated in Alzheimer's disease neuroimaging initiative (ADNI) participants with baseline mild cognitive impairment. Brain Imaging Behav. 2012;6:517–27.
38. Crane PK, Carle A, Gibbons LE, Insel P, Mackin RS, Gross A, et al. Development and assessment of a composite score for memory in the Alzheimer's disease neuroimaging initiative (ADNI). Brain Imaging Behav. 2012;6:502–16.
39. Choi SE, Mukherjee S, Gibbons LE, Sanders RE, Jones RN, Tommet D, et al. Development and validation of language and visuospatial composite scores in ADNI. Alzheimers Dement. 2020;6:e12072.
40. Huang L, Chen KL, Lin BY, Tang L, Zhao QH, Lv YR, et al. Chinese version of montreal cognitive assessment basic for discrimination among different severities of Alzheimer's disease. Neuropsychiatr Dis Treat. 2018;14:2133–40.
41. Karikari TK, Pascoal TA, Ashton NJ, Janelidze S, Benedet AL, Rodriguez JL, et al. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a

diagnostic performance and prediction modelling study using data from four prospective cohorts. Lancet Neurol. 2020;19:422–33.
42. Bittner T, Zetterberg H, Teunissen CE, Ostlund RE Jr., Militello M, Andreasson U, et al. Technical performance of a novel, fully automated electrochemiluminescence immunoassay for the quantitation of β-amyloid (1–42) in human cerebrospinal fluid. Alzheimers Dement. 2016;12:517–26.
43. Landau SM, Mintun MA, Joshi AD, Koeppe RA, Petersen RC, Aisen PS, et al. Amyloid deposition, hypometabolism, and longitudinal cognitive decline. Ann Neurol. 2012;72:578–86.
44. Landau SM, Harvey D, Madison CM, Koeppe RA, Reiman EM, Foster NL, et al. Associations between cognitive, functional, and FDG-PET measures of decline in AD and MCI. Neurobiol Aging. 2011;32:1207–18.
45. Minoshima S, Drzezga AE, Barthel H, Bohnen N, Djekidel M, Lewis DH, et al. SNMMI procedure standard/EANM practice guideline for amyloid PET imaging of the brain 1.0. J Nucl Med. 2016;57:1316–22.
46. Tian M, Zuo C, Civelek AC, Carrio I, Watanabe Y, Kang KW, et al. International nuclear medicine consensus on the clinical use of amyloid positron emission tomography in Alzheimer's disease. Phenomics. 2022;3:375–89.
47. Goldstein A, Kapelner A, Bleich J, Pitkin E. Peeking inside the black box: visualizing statistical learning with plots of individual conditional expectation. J Comput Graph Stat. 2015;24:44–65.
48. Rosipal R, Krämer N. Overview and recent advances in partial least squares. In: Saunders C, Grobelnik M, Gunn S, Shawe-Taylor J, editors. Subspace, latent structure and feature selection techniques. Berlin, Heidelberg: Springer; 2006, vol 3940 pp. 34–51.
49. Lopez-Lee C, Torres ERS, Carling G, Gan L. Mechanisms of sex differences in Alzheimer's disease. Neuron. 2024;112:1208–21.
50. Lopez-Lee C, Kodama L, Fan L, Zhu D, Zhu J, Wong MY, et al. Tlr7 drives sex differences in age- and Alzheimer's disease–related demyelination. Science. 2024;386:eadk7844.
51. Saraste M, Matilainen M, Vuorimaa A, Laaksonen S, Sucksdorff M, Leppert D, et al. Association of serum neurofilament light with microglial activation in multiple sclerosis. J Neurol Neurosurg Psychiatry. 2023;94:698–706.
52. Johnson NR, Yuan P, Castillo E, Lopez TP, Yue W, Bond A, et al. CSF1R inhibitors induce a sex-specific resilient microglial phenotype and functional rescue in a tauopathy mouse model. Nat Commun. 2023;14:118.
53. Cipolla MJ, Godfrey JA, Wiegman MJ. The effect of ovariectomy and estrogen on penetrating brain arterioles and blood-brain barrier permeability. Microcirculation. 2009;16:685–93.
54. Wilson AC, Clemente L, Liu T, Bowen RL, Meethal SV, Atwood CS. Reproductive hormones regulate the selective permeability of the blood-brain barrier. Biochim Biophys Acta. 2008;1782:401–7.
55. Parrado-Fernández C, Blennow K, Hansson M, Leoni V, Cedazo-Minguez A, Björkhem I. Evidence for sex difference in the CSF/plasma albumin ratio in ~20 000 patients and 335 healthy volunteers. J Cell Mol Med. 2018;22:5151–4.
56. Moon Y, Lim C, Kim Y, Moon WJ. Sex-related differences in regional blood-brain barrier integrity in non-demented elderly subjects. Int J Mol Sci. 2021;22:2860.
57. Palermo G, Mazzucchi S, Della Vecchia A, Siciliano G, Bonuccelli U, Azuar C, et al. Different clinical contexts of use of blood neurofilament light chain protein in the spectrum of neurodegenerative diseases. Mol Neurobiol. 2020;57:4667–91.

ACKNOWLEDGEMENTS

Data used in preparation of this article were partially obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wpcontent/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf. Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Cogstate; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California. We thank Dr. Wei Cheng (Fudan University) for his guidance on the bootstrap resample method for survival analysis.

AUTHOR CONTRIBUTIONS

PY, XQC designed the study. ZHW and YFX carried out the analysis. KH, YW, QHG,and FX directed the data collection in the validation cohort. PY, ZHW and YFX made the graphs. PY, XQC and FX wrote and edited the manuscript. PY directed the study.

FUNDING

This work was supported by the National Key Research and Development Program of China (2023YFC3603200, 2016YFC1306305, 2018YFE0203600), Shanghai Pilot Program for Basic Research - FuDan University 21TQ1400100 (22TQ019), Shanghai Municipal Science and Technology Major Project, the Lingang Laboratory (grant no. LG-QS-202203-09), Shanghai Natural Science Foundation (22ZR1415000), and STI2030-Major Projects (2022ZD0213800).

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL

The study of C-PAS cohort was approved by the Independent Ethics Committee "Huashan Hospital Institutional Review Board (HIRB), Fudan University" (approval number: KY-2017-406), and all participants gave written informed consent. All methods were performed in accordance with the relevant guidelines and regulations.

INFORMED CONSENT

We confirm that all human subjects provided informed consent in the study.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41380-025-03149-z.

Correspondence and requests for materials should be addressed to Fang Xie or Peng Yuan.