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RESEARCH ARTICLE

# Neurofilament light chain concentration mediates the association between regional medial temporal lobe structure and memory in adults with Down syndrome

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## Abstract

**INTRODUCTION:** Virtually all people with Down syndrome (DS) develop neuropathology associated with Alzheimer's disease (AD). Atrophy of the hippocampus and entorhinal cortex (EC), as well as elevated plasma concentrations of neurofilament light chain (NfL) protein, are markers of neurodegeneration associated with late-onset AD. We hypothesized that hippocampus and EC gray matter loss and increased plasma NfL concentrations are associated with memory in adults with DS.

**METHODS:** T1-weighted structural magnetic resonance imaging (MRI) data were collected from 101 participants with DS. Hippocampus and EC volume, as well as EC

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subregional cortical thickness, were derived. In a subset of participants, plasma NfL concentrations and modified Cued Recall Test scores were obtained. Partial correlation and mediation were used to test relationships between medial temporal lobe (MTL) atrophy, plasma NfL, and episodic memory.

**RESULTS:** Hippocampus volume, left anterolateral EC (alEC) thickness, and plasma NfL were correlated with each other and were associated with memory. Plasma NfL mediated the relationship between left alEC thickness and memory as well as hippocampus volume and memory.

**DISCUSSION:** The relationship between MTL gray matter and memory is mediated by plasma NfL levels, suggesting a link between neurodegenerative processes underlying axonal injury and frank gray matter loss in key structures supporting episodic memory in people with DS.

**KEYWORDS**

Alzheimer's disease, anterolateral entorhinal cortex, cognitive decline, dementia, Down syndrome, episodic memory, hippocampus, medial temporal lobe, mild cognitive impairment

## 1 | INTRODUCTION

Roughly 1 in 700 babies in the United States is born with Down syndrome (DS), a genetic disorder caused by a triplication of chromosome 21.[1] Trisomy 21 leads to overproduction of amyloid beta (A$\beta$) protein, causing pathological accumulation of fibrils mirroring genetic forms of Alzheimer's disease (AD).[2] By age 40, almost all people with DS develop A$\beta$ plaques and tau tangles, the hallmark pathologies of AD.[3] AD is the primary cause of neurodegeneration, dementia, and mortality in people with DS.[4,5] The estimated prevalence of dementia in people with DS is 23% at age 50, 45% at age 55, and 88% at age 65.[6]

Neurofilament light chain protein (NfL) is released into the extracellular space of the brain following axonal damage. Trace amounts of NfL are detectable in cerebrospinal fluid (CSF) and plasma.[7] CSF and plasma concentrations of NfL are strongly correlated.[8] Although the release of NfL is age related[9] and not specific to AD,[10] it is an emerging, minimally invasive biomarker of neurodegeneration in people with DS.

Synaptic and neuronal loss in the brain regions of interest can be measured indirectly with magnetic resonance imaging (MRI)–derived volumetric and cortical thickness estimates. The earliest neurodegenerative changes in AD and AD-DS occur in regions susceptible to pathology accumulation such as the hippocampus[11,12] and entorhinal cortex (EC).[13–16] The hippocampus is also subject to age-related neurodegeneration,[17,18] although no age-related neuron loss is observed within EC.[13] The association between EC thickness, AD pathology, and cognitive decline is driven by the anterolateral subregion of the EC (alEC).[15] This observation is consistent with neuropathological evidence of alEC vulnerability to early tau pathology.[19]

The EC and hippocampus are key brain regions that enable episodic memory.[20] The modified Cued Recall Test (mCRT) is used to assess verbal episodic memory in people with DS while accounting for developmental differences in cognitive ability.[21] We hypothesized that MRI-based measures of hippocampus and EC (in particular, alEC), gray matter, and plasma NfL concentration would be associated with each other as well as mild cognitive impairment (MCI)/Alzheimer's disease (AD) clinical status and memory in people with DS. To disentangle these hypothesized relationships, we performed a mediation analysis to explore the contributions of both neurodegenerative indicators to memory impairment.

## 2 | METHODS

### 2.1 | Participants

Participants enrolled in the Alzheimer's Disease in Down Syndrome (ADDS) study, which aims to characterize the evolution of AD in people with DS using neuropsychological assessments, blood, and neuroimaging biomarkers. The ADDS study enrolled over 200 people with DS age 40 and older at three sites: Columbia University (CU), Massachusetts General Hospital (MGH), and the University of California, Irvine (UCI). The current analyses included participants who completed an MRI scan. Some participants also completed memory assessments and had plasma samples collected. Consent Statement: All research procedures were reviewed and approved by the institutional review boards of all collaborating institutions, and informed consent, as well as assent, was obtained from all participants and their legally authorized representatives. All participants were reimbursed for their participation.

### 2.2 | MRI acquisition

Data were collected on Siemens Prisma 3T MRI scanners at all three institutions, in addition to a Philips 3T Achieva scanner at UCI, which was used until UCI transitioned to the Siemens scanner

mid-way through data collection. All scanners are equipped with 32-channel coils. A T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE) anatomic scan based on the Alzheimer's Disease Neuroimaging Initiative 3 (ADNI-3) parameters was collected on all participants (Siemens: sagittal acquisition, repetition time [TR] = 2300 ms, echo time [TE] = 2.96 ms, inversion time [TI] = 900 ms, flip angle = 9°, voxel resolution = 1.0 mm isotropic, field of view [FOV] = 256 × 256 mm, 208 slices; Philips: sagittal acquisition, TR = 7.800 ms, TE = 3.59, flip angle = 7°, voxel resolution = 1.0 mm isotropic, FOV = 256 × 256 mm, 176 slices).

## 2.3 | Structural imaging processing and analysis

Medial temporal lobe (MTL) cortex subregional segmentation was performed with a consensus labeling approach based on a set of 17 T2-weighted images acquired with an optimized MTL-specific acquisition protocol (image resolution: 0.47 × 0.47mm$^2$ in-plane, 2.0 mm slice thickness) from cognitively normal neurotypical participants who were manually labeled using a highly reliable anatomic protocol used in prior published work.[22,23] Anatomic labeling of the atlas set comprises separate labels for left and right EC, alEC, and posteromedial EC (pmEC). The alEC and pmEC boundaries were based on segmentations used in Reagh et al.[24] Scans were paired with corresponding T1-weighted images (image resolution: 0.75 × 0.75 × 0.75mm$^3$) acquired for multi-spectral atlas-based registration. Spatial normalization and registration of the images to the atlas were performed (see Supplementary Methods). Visual quality assessment was performed to exclude participants with excessive motion artifacts or errors in registration or segmentation (see Supplementary Methods).

Voxels within bilateral EC, alEC, and pmEC were counted and multiplied by voxel resolution to calculate volumes in cubic millimeters. Cortical thickness of left and right alEC and pmEC was calculated by dividing volume by surface area for all regions. Cortical reconstruction was performed using Freesurfer 6.0[25] to obtain whole hippocampus volume as well as total intracranial volume (ICV). All volumes were normalized by dividing each participant's measure by their ICV. Regions of interest are shown in Figure 1A.

## 2.4 | Memory assessment

Episodic memory was assessed using a modified version of the Cued Recall Test (or mCRT) validated for use in people with intellectual disability. The mCRT is sensitive to preclinical and clinical AD in people with DS.[26,27] Stimuli were arranged on three cards, each with four line drawings of everyday objects from unique semantic categories, totaling 12 items. During encoding, participants were shown one card at a time and asked to identify which item was a member of a cued category. The card was removed, and participants were asked to recall the item. This was repeated for the remaining 11 items. During retrieval, participants were asked to freely recall all 12 items. This was followed by cued recall of any forgotten items using the category cue. Correct responses were recorded as the free recall score (FRS) and cued recall score (CRS), respectively. FRS and CRS were repeated for two additional trials. The total recall score (TRS) was the sum of FRS and CRS across the three trials, resulting in a maximum score of 36. Due to the dependence of CRS on FRS, only FRS and TRS were used as memory outcome measures.

### RESEARCH IN CONTEXT

1. **Systematic review**: People with Down syndrome (DS) have elevated risk for Alzheimer's disease (AD). The authors reviewed the literature on neurodegeneration in AD-DS in PubMed, paper preprint archives like bioRxiv, and current research from the Alzheimer's Biomarkers Consortium–Down Syndrome (ABC-DS). This manuscript addresses the vulnerability of medial temporal lobe cortical thickness and volume to neurodegeneration in AD-DS and how such atrophy may be related to plasma measures of axonal injury.
2. **Interpretation**: Our findings suggest that anterolateral entorhinal cortical thinning, hippocampus volume loss, and elevated plasma neurofilament light concentrations are biological features associated with dementia in people with DS and may be part of a common pathway for memory loss in AD-DS.
3. **Future directions**: Future work will examine the relationship between these neurodegenerative processes and amyloid and tau positron emission tomography and will include longitudinal follow-up to assess whether these measures predict cognitive decline.

## 2.5 | Determination of MCI/AD clinical status

Each participant's MCI/AD clinical status was evaluated with a standardized assessment battery (see Supplementary Methods). Following data collection, the MCI/AD clinical status of each participant was assigned at a consensus case conference conducted without knowledge of mCRT, neuroimaging, or fluid measures.[28] Consensus diagnosis was performed in accordance with the International Classification of Diseases 10th Revision and the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision criteria described in Sheehan et al.[29] MCI/AD clinical status was classified into the following categories: *(a) cognitively stable (CS)*, indicating with reasonable certainty that clinically significant declines beyond those to be expected with normal aging were absent; *(b) mild cognitive impairment (MCI-DS)*, indicating that there was some mild cognitive and/or functional decline greater than would be expected with aging but not severe enough to meet criteria for dementia; *(c) possible dementia*, indicating significant decline over 6 months or more that suggested dementia was likely, but additional information was needed to establish certainty; *(d)*

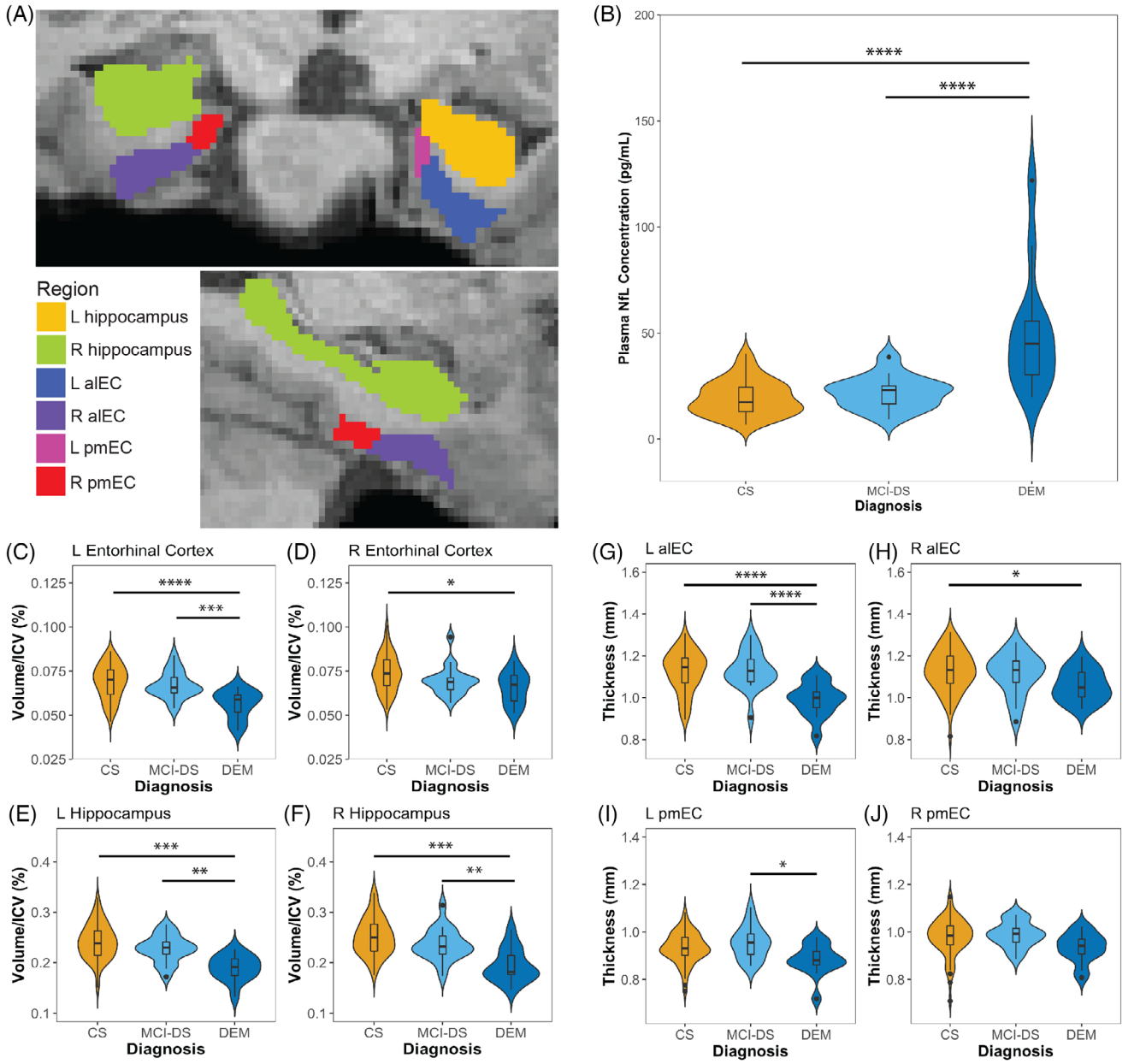


**FIGURE 1** (A) Regions of interest. (B) Plasma NfL concentration was significantly higher in the DEM group compared with the CS and MCI-DS groups. (C) The DEM group had lower left EC volume than the MCI-DS and CS groups. (D) The DEM group also had lower right EC volume than the CS group but not the MCI-DS group. (E, F) Left and right hippocampus volume was lower in the DEM group compared with the MCI-DS and CS groups. (G) The DEM group had lower left alEC thickness than the MCI-DS and CS groups. (H) The DEM group also had lower right alEC thickness than the CS group but not the MCI-DS group. (I) The DEM group had lower left pmEC thickness than the CS group. (J) Right pmEC thickness did not differ between the three groups. NfL, neurofilament light; ICV, intracranial volume; L, left; R, right; EC, entorhinal cortex; alEC, anterolateral entorhinal cortex; pmEC, posteromedial entorhinal cortex; CS, cognitively stable; MCI-DS = mild cognitive impairment–Down syndrome; DEM, dementia. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

*definite dementia*, indicating a high degree of confidence that dementia was present; or *(e) status uncertain due to complications*, indicating that the evidence of decline was present but one or more factors unrelated to an aging-associated neuropathology might be the cause, usually a medical condition or traumatic life event. In most cases, a consensus determination was straightforward (see Supplementary Methods). For the purposes of analysis, participants with possible and definite dementia were collapsed into a single dementia (DEM) group.

## 2.6 | Blood sample collection and plasma NfL processing

The participants were not required to fast before blood draw. Blood samples were collected with a butterfly catheter. Within 30 min of blood collection, the tubes were centrifuged at the appropriate speed and temperature to separate out plasma, stored in separate 250 $\mu$L polypropylene aliquots, and frozen at −80°C. NfL chain concentrations

**TABLE 1** Participant demographics and neuropsychological assessments for individuals with both MRI and mCRT data.

| Variable | CS | MCI-DS | DEM | Test statistic (*F* value or $\chi^2$) | Group differences |
|---|---|---|---|---|---|
| *N* | 64 | 18 | 10 | n/a | n/a |
| Age (mean ± SD) | 48.7 ± 5.8 | 52.2 ± 5.2 | 54.9 ± 7.5 | 6.3 | B |
| Female (%) | 25 (37.5%) | 5 (27.8%) | 5 (50.0%) | 1.4 | n.s. |
| mCRT FRS | 16.9 ± 5.8 | 10.8 ± 5.7 | 5.8 ± 6.8 | 20.0 | AB |
| mCRT TRS | 31.0 ± 6.9 | 22.7 ± 10.5 | 10.6 ± 10.2 | 30.7 | ABC |

*Note*: Significant pairwise group differences, $p < 0.05$: A: CS versus MCI-DS; B: CS versus DEM; C: MCI-DS versus DEM.
Abbreviations: CS, cognitively stable; DEM, dementia; FRS, free recall score; MCI-DS, mild cognitive impairment–Down syndrome; mCRT, modified Cued Recall Test; SD, standard deviation; TRS, total recall score.

were determined with the Single Molecule Array (Simoa) technology using the Human NF-Light Advantage kit (Simoa; Quanterix, Lexington, MA, USA) completed at the University of North Texas Health Science Center, Institute for Translational Research. Further information regarding NfL sample processing and performance parameters can be found in Petersen et al.[30]

## 2.7 | Statistical analysis

All data analyses were conducted using R[31] (v3.5.1) and RStudio[32] (v1.1.447) software. Normality was assessed visually using quantile–quantile plots and statistically using the Shapiro–Wilk test. All variables of interest were normally distributed except for TRS, right pmEC thickness, and plasma NfL. TRS could not be transformed to approximate a normal distribution, but it is included as an outcome variable based on the recommendation by Krinsky-McHale et al.[21] When right pmEC thickness and plasma NfL were log-transformed, the results were similar to the untransformed variables (see Supplementary Results), so the latter were used during analysis. All models included age, sex, and site as covariates. Differences in demographic and neuropsychological variables among the diagnosis groups (CS, MCI-DS, and DEM) were tested using one-way analysis of variance (ANOVA) with post hoc Tukey test or chi-square test for continuous and categorical measures, respectively. Diagnosis group differences in MRI measures and plasma NfL concentration were tested using multiple linear regression and pairwise contrasts. Effect size for regressions was calculated using the *emmeans* package in R[33] (v1.7.5). Associations between MRI measures, plasma NfL concentration, and memory performance were tested using Pearson partial correlation. Reported results were considered significant at $\alpha < 0.05$, after correcting for multiple comparisons using the Holm method[34] Two participants in the DEM group had plasma NfL concentrations > 3 SD above the mean. Sensitivity analyses excluding these participants were conducted for all statistical tests involving plasma NfL, and the results remained the same. Causal mediation analysis was performed using the *mediation* package in R[35] (v4.5.0). One thousand bootstrap samples were used to calculate bias-corrected and accelerated confidence intervals (CIs) at the 2.5th and 97.5th percentiles.

# 3 | RESULTS

## 3.1 | Participant demographics and neuropsychological assessments

A total sample of 101 participants had MRI data, after excluding participants who had uncertain diagnoses ($n = 11$), whose scans had excessive motion ($n = 26$), and whose scans did not pass processing quality control procedures ($n = 11$). Demographic information for this sample is summarized in Table S1. Of those 101 participants, 92 had mCRT data. Demographic information and neuropsychological testing results for this primary sample are summarized in Table 1. The distribution of participants across sites was as follows: CU $n = 23$; MGH $n = 29$; UCI $n = 40$. Participants from each diagnosis group were distributed proportionately across the three sites ($\chi^2 = 3.3904$, $p = 0.4947$). Signal-to-noise ratio (SNR) and other quality control measures across sites were comparable. Among the 101 participants with MRI data, 86 had plasma NfL data. Their demographic information is summarized in Table S2. A total of 77 participants had MRI, plasma NfL, and mCRT. Their demographic information and neuropsychological testing results are summarized in Table S3.

## 3.2 | Increased plasma NfL concentration in dementia group

Among participants with MRI and plasma NfL data, plasma NfL concentration was higher in the DEM group compared with the MCI-DS ($t(78) = -4.709$, $p < 0.0001$, effect size = −1.922) and CS groups ($t(78) = -5.907$, $p < 0.0001$, effect size = −2.034) (Figure 1B). There was no difference in plasma NfL concentration between the CS and MCI-DS groups ($t(78) = -0.370$, $p = 0.7128$, effect size = −0.112).

## 3.3 | Reduced EC and hippocampus volume in dementia group

Left EC volume was lower in the DEM group compared with the MCI-DS ($t(93) = 3.746$, $p = 0.0003$, effect size = 1.391) and CS groups ($t(93) = 4.628$, $p < 0.0001$, effect size = 1.518) (Figure 1C). There was no difference in left EC volume between the CS and MCI-DS groups

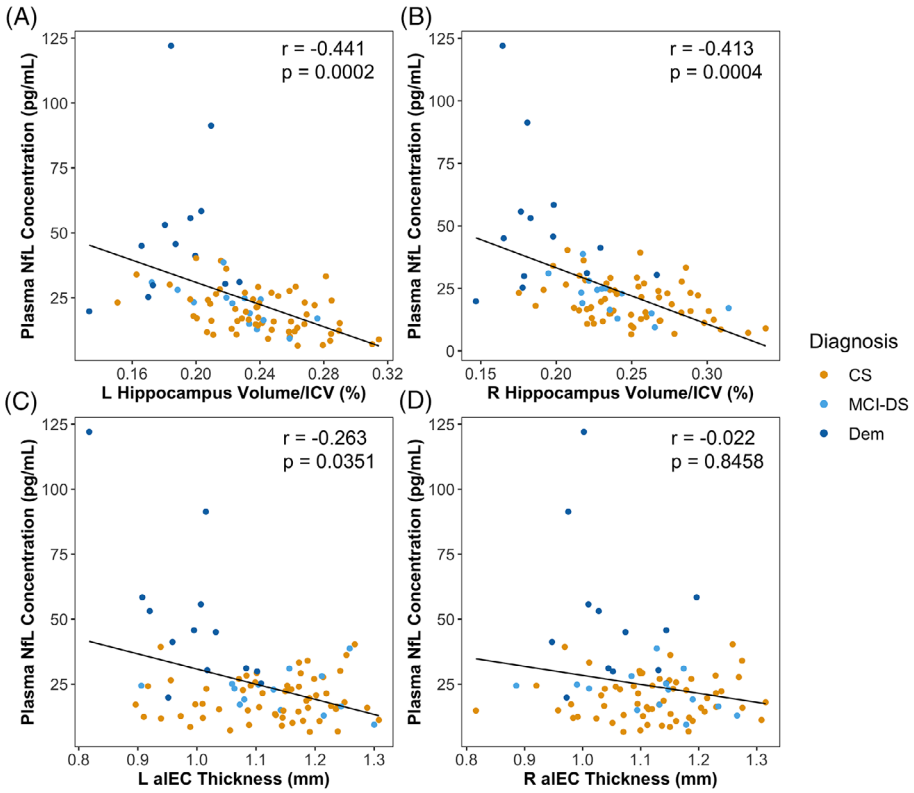


**FIGURE 2** (A–C) Plasma NfL concentration was negatively associated with bilateral hippocampus volume/ICV percentage and left alEC thickness. (D) Plasma NfL levels were not associated with right alEC thickness. Pearson's *r* is the partial correlation coefficient after controlling for age, sex, and site covariates. The plots include the two outliers with high plasma NfL concentration, since their exclusion did not meaningfully alter the results. NfL, neurofilament light; ICV, intracranial volume; alEC, anterolateral entorhinal cortex L, left; R, right; CS, cognitively stable; MCI-DS, mild cognitive impairment–Down syndrome; DEM, dementia.

($t(93) = 0.477$, $p = 0.6344$, effect size = 0.128). Right EC volume was lower in the DEM group compared with the CS group ($t(93) = 2.354$, $p = 0.0207$, effect size = 0.772), but there was no difference in right EC volume between the DEM and MCI-DS groups ($t(93) = 1.203$, $p = 0.2322$, effect size = 0.447) or between the MCI-DS and CS groups ($t(93) = 1.219$, $p = 0.2261$, effect size = 0.326) (Figure 1D).

The DEM group had lower left and right hippocampus volume than the MCI-DS (left: $t(93) = 2.704$, $p = 0.0082$, effect size = 1.004; right: $t(93) = 2.596$, $p = 0.0109$, effect size = 0.964) and CS groups (left: $t(93) = 3.827$, $p = 0.0002$, effect size = 1.256; right: $t(93) = 3.884$, $p = 0.0002$, effect size = 1.274) (Figure 1E,F). There was no difference in hippocampus volume between the CS and MCI-DS groups (left: $t(93) = 0.942$, $p = 0.3488$, effect size = 0.252; right: $t(93) = 1.160$, $p = 0.2490$, effect size = 0.310).

## 3.4 | Reduced alEC and pmEC thickness in the dementia group

Following our recent work in ADNI,[15] we sought to determine whether the EC volumetric differences across diagnosis groups may be driven by its anterolateral or posteromedial segments. Left alEC thickness was lower in the DEM group compared with the MCI-DS ($t(93) = 4.532$, $p < 0.0001$, effect size = 1.683) and CS groups ($t(93) = 4.939$, $p < 0.0001$, effect size = 0.618) (Figure 1G). There was no difference in left alEC thickness between the CS and MCI-DS groups ($t(93) = -0.242$, $p = 0.8094$, effect size = −0.065). Right alEC thickness was lower in the DEM group compared with the CS group ($t(93) = 2.190$, $p = 0.0311$, effect size = 0.718) (Figure 1H). There were no differences in right alEC thickness between the DEM and MCI-DS groups ($t(93) = 1.504$, $p = 0.1359$, effect size = 0.559) or between the MCI-DS and CS groups ($t(93) = 0.598$, $p = 0.5515$, effect size = 0.160).

Left pmEC thickness was lower in the DEM group compared with the MCI-DS group ($t(93) = 2.176$, $p = 0.0321$, effect size = 0.808) (Figure 1I). The difference in left pmEC thickness between the DEM and CS groups was trending toward significant ($t(93) = 1.685$, $p = 0.0953$, effect size = 0.553), whereas the difference between the MCI-DS and CS groups was not significant ($t(93) = -0.954$, $p = 0.3425$, effect size = −0.255). Right pmEC thickness showed no differences between any of the groups (all *p*-values > 0.1) (Figure 1J).

## 3.5 | Left alEC thickness and bilateral hippocampus volume are associated with plasma NfL concentration

Among participants with MRI and plasma NfL data, there was an inverse relationship between plasma NfL concentration and left

hippocampus volume ($r = -0.337$, $p = 0.0037$), right hippocampus volume ($r = -0.432$, $p = 0.0002$), and left alEC thickness ($r = -0.403$, $p = 0.0005$) but not right alEC thickness ($r = -0.178$, $p = 0.1067$) (Figure 2). There was no statistically significant partial correlation between plasma NfL concentration and left or right pmEC thickness, even after excluding the two outliers (all $p$-values > 0.2) (Figure S1).

### 3.6 | Hippocampus volume, alEC thickness, and plasma NfL concentration are associated with memory recall

Among participants with MRI and mCRT data, there were significant positive partial correlations between both memory scores and left hippocampus volume (FRS: $r = 0.352$, $p = 0.0021$; TRS: $r = 0.393$, $p = 0.0006$) and right hippocampus volume (FRS: $r = 0.380$, $p = 0.0010$; TRS: $r = 0.377$, $p = 0.0008$), as well as trending correlations for left alEC thickness (FRS: $r = 0.211$, $p = 0.0944$; TRS: $r = 0.234$, $p = 0.0540$) and right alEC thickness (FRS: $r = 0.196$, $p = 0.0944$; TRS: $r = 0.193$, $p = 0.0704$) (Figure 3). There were no statistically significant partial correlations between either memory score and bilateral pmEC thickness (all $p$-values > 0.2) (Figure S2).

Among participants with MRI, plasma NfL, and mCRT data, there were significant negative partial correlations between plasma NfL and FRS ($r = -0.438$, $p < 0.0001$) and TRS ($r = -0.475$, $p < 0.0001$) (Figure 4).

### 3.7 | Plasma NfL mediates the relationships between left alEC thickness and memory recall

To further understand the potential directional relationships among left alEC thickness and bilateral hippocampus volume, plasma NfL, and memory performance, we used a mediation analysis and reduced our sample to participants who had all these measures ($n = 77$). Because FRS and TRS are highly correlated, we chose to test only FRS as an outcome variable.

Thinner left alEC was associated with greater plasma NfL concentration ($a = -34.551$, $t(72) = -2.507$, $p = 0.0144$), which in turn was associated with worse FRS ($b = -0.216$, $t(71) = -3.534$, $p = 0.0007$). There was an indirect effect of plasma NfL on the relationship between left alEC thickness and FRS ($ab = 7.464$, 95% CI = 0.902, 16.9; $p = 0.024$) (Figure 5). After adjusting for plasma NfL, the direct effect of left alEC thickness on FRS was not significant ($c' = 6.397$, 95% CI = −6.363, 20.6; $p = 0.384$). Plasma NfL mediated 54% of the relationship between left alEC thickness and FRS.

When the reverse mediation was run, with left alEC thickness as the mediator between plasma NfL and FRS, there was no indirect effect of left alEC thickness on the relationship between plasma NfL and FRS ($ab = -0.0149$, 95% CI = −0.0551, 0.01; $p = 0.352$), with left alEC thickness mediating only 6% of the relationship between plasma NfL and FRS (Figure S3).

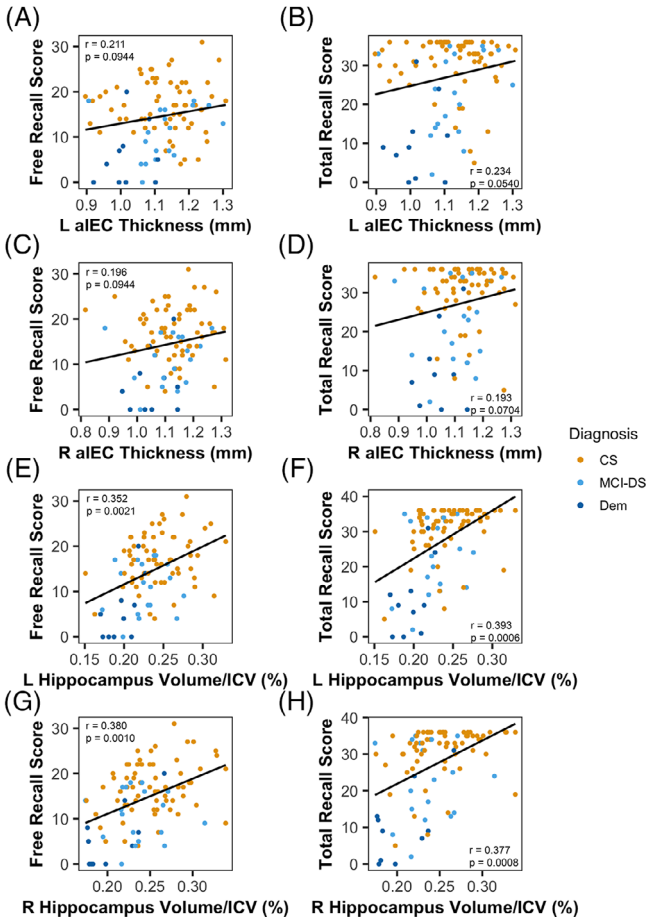


**FIGURE 3** Hippocampus volume/ICV percentage and alEC thickness were positively associated with FRS and TRS. Pearson's $r$ is the partial correlation coefficient after controlling for age, sex, and site covariates. (A–D) Positive partial correlations between FRS and TRS and left and right hippocampus volume were significant. (E–H) Positive partial correlations between the two memory scores and alEC thickness were trending toward significant. FRS, free recall score; TRS, total recall score; alEC, anterolateral entorhinal cortex; L, left; R, right, ICV, intracranial volume; CS, cognitively stable; MCI-DS, mild cognitive impairment–Down syndrome; DEM, dementia.

We repeated the mediation analyses described above for left and right hippocampus volume (Figure S4). Plasma NfL partially mediated the relationship between left and right hippocampus volume on FRS, but left and right hippocampus volume also partially mediated the relationship between plasma NfL and FRS.

## 4 | DISCUSSION

The present study hypothesized that plasma NfL concentration and EC and hippocampus gray matter atrophy would be associated with dementia and memory in people with DS. Individuals with dementia had higher plasma NfL concentrations, lower bilateral hippocampus volume, and lower left alEC thickness than CS and MCI-DS individuals. No differences in plasma NfL levels or MRI measures were observed

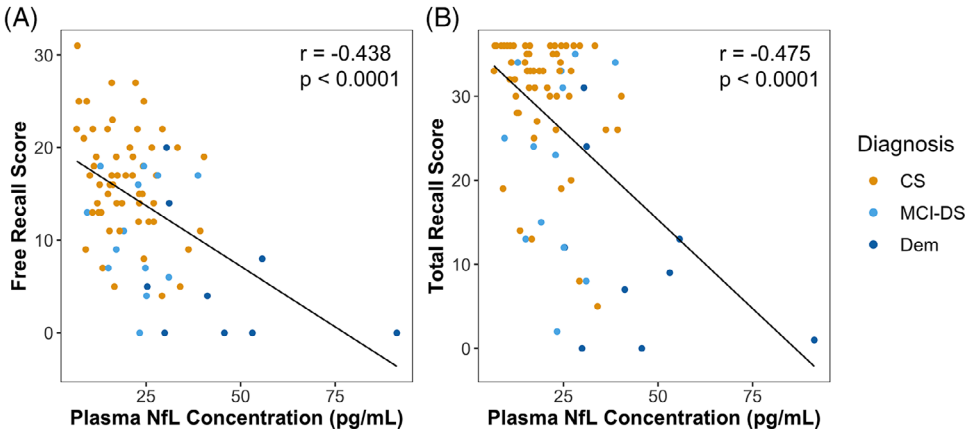


**FIGURE 4** Plasma NfL concentration was negatively associated with FRS (A) and TRS (B). Pearson's *r* is the partial correlation coefficient after controlling for age, sex, and site covariates. The plots include the outlier with high plasma NfL concentration, since the exclusion of this participant did not meaningfully alter the results. NfL, neurofilament light; FRS, free recall score; TRS, total recall score; CS, cognitively stable; MCI-DS, mild cognitive impairment–Down syndrome; DEM, dementia.

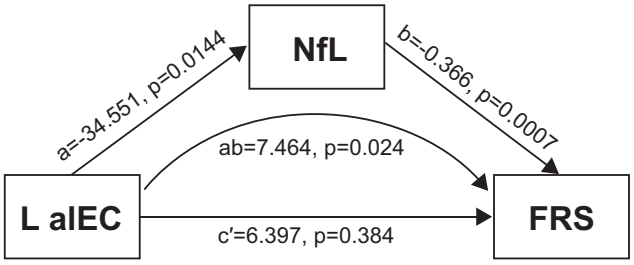


**FIGURE 5** Plasma NfL concentration mediated the relationship between left alEC thickness and FRS. The indirect effect of plasma NfL on the relationship between left alEC thickness and FRS represented by ab was significant. The direct effect of left alEC thickness on FRS, represented by c′, was not significant after accounting for plasma NfL. NfL, neurofilament light; alEC, anterolateral entorhinal cortex; L, left; FRS, free recall score.

between CS and MCI-DS individuals. Our findings replicate previous work showing that plasma NfL differentiates asymptomatic individuals with DS from those with clinical AD.[36] Lower hippocampus volume has been observed in CS individuals with DS who are A$\beta$ positive[37] and in those who convert to MCI-DS.[38] We did not observe these early changes in hippocampus volume. There are age-related differences in hippocampus volume and cortical thickness in people with DS, although the EC shows no such difference.[18,39] Removing age as a covariate had a negligible effect on the results of our analysis (see Supplementary Results). Our findings suggest that the hippocampus and EC are vulnerable to neurodegeneration in people with DS with symptomatic AD. We believe our study is the first to demonstrate differential susceptibility to EC thinning in the left hemisphere and along its anterolateral/posteromedial axis in people with DS with dementia.

Left alEC thickness, bilateral hippocampus volume, and plasma NfL concentration were associated with free and total recall. These results are consistent with studies showing that verbal episodic memory is positively associated with hippocampus volume[38] and EC thickness[40] and negatively associated with plasma NfL levels[36] in individuals with DS across the AD spectrum. Plasma NfL levels were negatively correlated with bilateral hippocampus volume and left alEC thickness but not with other EC subregions, in line with findings in neurotypical individuals with and without symptomatic AD.[8,41-43] In sporadic AD, gray matter loss occurs earlier and progresses faster in the left hemisphere.[44] This asymmetric vulnerability to neurodegeneration does not appear to be unique to people with DS. Our findings show that focal neurodegeneration in the hippocampus and left alEC is linked to impaired episodic memory in older individuals with DS. Despite being a peripheral measure, plasma NfL concentration has a strong, anatomically specific relationship with hippocampus and left alEC neurodegeneration and tracks with episodic memory. The present study is among the first to establish a relationship between plasma NfL and MRI-based measures of neurodegeneration in people with DS.

Plasma NfL levels mediated the relationship between left alEC thickness and memory. Surprisingly left alEC thickness did not mediate the relationship between plasma NfL and memory. In contrast, plasma NfL partially mediated the relationship between bilateral hippocampus volume and memory and vice versa. This suggests that the impact of left alEC thickness on verbal episodic memory may be indirect and mediated by the production and release of NfL, whereas hippocampus volume and NfL each have a direct impact on memory. It is not clear whether plasma NfL concentrations increase downstream of gray matter loss. In neurotypical individuals across the AD spectrum, higher baseline plasma NfL predicts faster EC thinning and hippocampal volume loss, but smaller baseline EC thickness and hippocampus volume also predict a faster increase in plasma NfL levels.[41] Wallerian degeneration, or anterograde axonal degeneration down the length of the axon, is hypothesized to occur following gray matter loss.[45] The release of NfL into CSF and blood occurs as a direct result of axon degeneration.[46] Given that hippocampus and EC atrophy begin early in AD, initial increases in plasma NfL may reflect changes that are downstream to gray matter loss. However, the present study is not longitudinal so we cannot determine which comes first, an increased plasma NfL levels or atrophy in alEC and/or hippocampus.

This study has several limitations. We did not adjust for severity of intellectual disability, which could impact cognitive performance independent of dementia.[47] The sample size is relatively small due to missing data and is imbalanced across the diagnosis groups. Cross-sectional analysis cannot be used to establish causation or track an individual's trajectory over time. Future studies should be conducted on larger data sets to increase statistical power and facilitate longitudinal analysis. Amyloid and tau PET would enable in vivo mapping of neurodegeneration to the spatial pattern of pathology accumulation. Neuropathology studies of NfL could indicate the origin locations of peripherally-measured NfL and whether they overlap with brain regions vulnerable to AD-related neurodegeneration.

Our findings reveal that left alEC thickness and hippocampus volume reductions measured using MRI and increased levels of plasma NfL contribute to memory impairment in DS through shared neurodegenerative pathways. We highlight the left alEC as a brain region that is equally vulnerable to AD-related neurodegeneration as its better-studied hippocampal counterpart. Taken together, these data further our understanding of the pathophysiology underlying clinically relevant region-specific neurodegeneration in people with DS.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest relevant to this manuscript.

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## SUPPORTING INFORMATION

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