Cerebrospinal Fluid Biomarkers of Neurodegeneration, Synaptic Dysfunction, and Axonal Injury Relate to Atrophy in Structural Brain Regions Specific to Alzheimer's Disease

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Abstract

Introduction: Patterns of atrophy can distinguish normal cognition from Alzheimer's disease (AD), but neuropathological drivers of this pattern are unknown. This study examined associations between cerebrospinal fluid biomarkers of AD pathology, synaptic dysfunction, and neuroaxonal injury with two AD imaging signatures.

Methods: Signatures were calculated using published guidelines. Linear regressions related each biomarker to both signatures, adjusting for demographic factors. Bootstrapped analyses tested if associations were stronger with one signature versus the other.

Results: Increased phosphorylated tau (p-tau), total tau, and neurofilament light (P-values <.045) related to smaller signatures (indicating greater atrophy). Diagnosis and sex modified associations between p-tau and neurogranin (P-values<.05) and signatures, such that associations were stronger among participants with mild cognitive impairment and female participants. The strength of associations did not differ between signatures.

Discussion: Increased evidence of neurodegeneration, axonopathy, and tau phosphorylation relate to greater AD-related atrophy. Tau phosphorylation and synaptic dysfunction may be more prominent in AD-affected regions in females.

Key Words: AD signature, cortical thickness, cerebrospinal fluid, sex differences

Significance Statement: Certain patterns of brain atrophy on MRI distinguish individuals with normal cognition from clinical Alzheimer's disease (AD), but the neuropathological drivers of this atrophy pattern are unknown. We present novel neuroimaging and biomarker evidence suggesting that in addition to phosphorylated tau, axonopathy uniquely contributes to AD-related atrophy. Sex modifies associations linking both phosphorylated tau and synaptic dysfunction with atrophy in regions susceptible to AD-related neurodegeneration, such that associations are only present in females. Results suggest neuronal dysfunction may be more prominent in these regions susceptible to AD-related neurodegeneration among females and underscore the importance of examining sex-specific drivers of neuropathology and structural brain changes in AD.

1. Introduction

Alzheimer's disease (AD) is traditionally considered a disease of grey matter neurodegeneration, resulting from the accumulation of amyloid beta (Aβ) plaques and phosphorylated tau (p-tau).[1] As pathology evolves, atrophy occurs in a regionally specific pattern beginning in the temporal lobe and hippocampus[2] and spreading in concentric circles to the surrounding cortices. Recent efforts have identified magnetic resonance imaging (MRI) patterns of neurodegeneration that are specific to AD-related changes. Prior work shows that cortical thinning in the temporal and inferior parietal lobes quantified on T_1-weighted MRI can reliably predict cognitive decline[3] and conversion to AD.[4,5] Two identified patterns (the Schwarz AD signature[6] and the McEvoy AD signature[7]) reliably distinguish individuals with normal cognition from clinical AD. However, the neuropathological drivers of this regionally specific atrophy remain uncharacterized.

Prior work has found neurodegeneration within AD signature regions relate to amyloid deposition,[8] *in vivo* cerebrospinal fluid (CSF) measurements of p-tau and total tau (t-tau),[9] and tau burden at autopsy.[6] However, beyond core AD pathology, it is unknown how concomitant pathways to neurodegeneration, such as synaptic dysfunction or axonal injury, relate to atrophy in AD signature regions. Previous work has implicated neurogranin (reflecting synaptic degeneration[10]) and neurofilament light (NFL, a marker of axonal injury[11]) in AD, and each is associated with smaller hippocampal volumes.[11,12] Thus, these pathologies may contribute to atrophy detected in the AD signature. Further, evidence suggests that AD risk factors, such as female sex,[13] apolipoprotein E (*APOE*) ε4 carrier status,[14] and older age,[15] may lead to

heterogeneity in AD-related atrophy and must be considered when examining neuropathologic correlates of atrophy. For example, females with AD pathology have greater hippocampal atrophy compared to males,[13] and *APOE*-ε4 carriers have smaller hippocampal volumes compared to non-carriers.[14] To optimize the diagnostic and prognostic utility of AD signatures, associations of co-occurring pathologies, as well as sex-specific, genetic, and normal aging contributions, to clinically relevant atrophy must be elucidated.

The aim of this study is to investigate associations between CSF biomarkers of core AD pathology (Aβ and p-tau), neurodegeneration (t-tau), synaptic dysfunction (neurogranin), and axonal injury (NFL) with two publicly available AD neuroimaging signatures among older adults.[6,7] We hypothesize that increased CSF evidence of core AD pathology will relate to cortical thinning in AD signature regions, consistent with prior work,[9,16] and concomitant pathologies (i.e., neurodegeneration, synaptic dysfunction, and axonal injury) will independently contribute to this atrophy pattern beyond AD pathology. Given the imaging signature can differentiate individuals with and without cognitive impairment[6,7] and the known modifying effects of sex,[13] *APOE*-ε4 carrier status,[14] and age[15] on neurodegeneration, associations will be tested for interactions with diagnosis, sex, *APOE*-ε4 carrier status, and age. We hypothesize that associations between CSF biomarkers and the AD signature will be stronger in MCI participants, females, *APOE*-ε4 carriers, and older adults. Finally, we formally tested if the pattern of neurodegeneration associated with each CSF biomarker was more strongly related to one AD signature versus the other.

2. Materials & Methods

2.1 Study Cohort

The Vanderbilt Memory & Aging Project (VMAP) is a longitudinal observational study investigating vascular health and brain aging,[17] enriched with older adults with mild cognitive impairment (MCI). Inclusion required participants be ≥60 years, speak English, have adequate auditory and visual acuity, and have a reliable study partner. As part of a comprehensive screening, participants were excluded for a cognitive diagnosis of dementia,[18,19] MRI contraindication, history of neurological disease (e.g., multiple sclerosis, stroke), heart failure, major psychiatric illness, head injury with loss of consciousness>5 minutes, or a systemic or terminal illness affecting follow-up participation. At enrollment, participants completed a comprehensive examination, including (but not limited to) fasting blood draw, physical examination, clinical interview, medication review, echocardiogram, cardiac magnetic resonance, multi-modal brain MRI, and optional lumbar puncture. Participants were excluded from the current study for missing usable CSF, brain MRI, or covariate data. See **Figure 1** for inclusion/exclusion details. The protocol was approved by the Vanderbilt University Medical Center Institutional Review Board. Written informed consent was obtained from participants prior to data collection.

2.2 Lumbar Puncture & Biochemical Analysis

A subset of participants completed an optional fasting lumbar puncture at enrollment (n=153, **Figure 1**). CSF was collected with polypropylene syringes using a Sprotte 25-gauge spinal needle in an intervertebral lumbar space. Samples were

immediately mixed and centrifuged. Supernatants were aliquoted in 0.5 mL polypropylene tubes and stored at -80°C. Samples were analyzed in single batch using a commercially available enzyme-linked immunosorbent assays (Fujirebio, Ghent, Belgium) to measure CSF concentrations of Aβ42 (INNOTEST® β-AMYLOID$_{(1-42)}$), p-tau (INNOTEST® PHOSPHO-TAU$_{(181P)}$), and t-tau (INNOTEST® hTAU). Additional enzyme-linked immunosorbent assays measured CSF neurogranin[20] and CSF NFL (UmanDiagnostics, Umeå, Sweden) concentrations. Board-certified laboratory technicians processed data blinded to clinical information. Intra-assay coefficients of variation were <10%.[20]

2.3 APOE-ε4 Genotyping

APOE-ε4 genotyping was performed on deoxyribonucleic acid extracted from whole blood with a Taqman single-nucleotide polymorphism genotyping assay from Applied Biosystems (Foster City, California), as previously published.[17] Real-time polymerase chain reaction in 5 µl aliquots was performed on the Life Technologies 7900HT machine and results were analyzed using Life Technologies SDS 2.4.1 software. *APOE*-ε4 status was defined as carrier (ε2/ε4, ε3/ε4, ε4/ε4) or non-carrier (ε2/ε2, ε2/ε3, ε3/ε3).

2.4 Brain MRI Acquisition & Post-Processing

VMAP participants were scanned at the Vanderbilt University Institute of Imaging Science on a 3T Philips Achieva system (Best, the Netherlands) using an 8-channel SENSE reception coil array. T_1-weighted images (repetition time=8.9ms, echo

time=4.6ms, spatial resolution=1x1x1mm^3) were acquired as part of a larger multi-modal protocol and post-processed using FreeSurfer (http://surfer.nmr.mgh.harvard.edu/).[21] T_1-weighted images were registered to MNI space, intensity corrected, and skull stripped. Subcortical structures, cortical structures, and white matter were segmented, and white and grey matter surfaces were constructed for each hemisphere. These surfaces were used to calculate cortical thickness and inflated for visualization. Surfaces were manually inspected and corrected for registration, topological, and segmentation defects. After manual correction, images were reprocessed to update the transformation template and segmentation information. The Schwarz AD signature was calculated by summing bilateral cortical thickness measurements from regions shown to distinguish individuals with AD from normal cognition, including the entorhinal cortex, middle temporal cortex, inferior parietal cortex, fusiform gyrus, and precuneus.[6] The McEvoy AD signature was calculated by a weighted sum of volumes and cortical thickness measurements from regions shown to predict conversion to AD, including the hippocampus, entorhinal cortex, middle temporal cortex, superior temporal cortex, cingulate gyrus, and orbitofrontal gyrus.[7] Consistent with prior literature, contributions of intracranial volume were regressed out of the hippocampal volume term and contributions of age and sex were regressed out of the McEvoy signature.[7]

2.5 Experimental Design & Statistical Analyses

Linear regression models with ordinary least square estimates related continuous CSF measures of Aβ, p-tau, t-tau, neurogranin, and NFL concentrations (pg/mL) to each AD signature, adjusting for age, sex, race/ethnicity, education, *APOE*-ε4 status, and

cognitive diagnosis. Models examining the McEvoy AD signature also adjusted for intracranial volume. Linear regression models also related those CSF biomarkers with significant associations to either AD signature as competing predictors using identical covariates. This competitive model approach assessed the extent to which variance accounted for by an individual biomarker was unique or overlapping with another biomarker. Secondary models evaluated *CSF biomarker x cognitive diagnosis*, *CSF biomarker x sex*, *CSF biomarker x APOE-ε4 status,* and *CSF biomarker x age* interactions on the AD signature. Models were repeated stratified by diagnosis (NC, MCI), sex (male, female), *APOE*-ε4 status (carrier, non-carrier), and age group based on the sample median (<73, ≥73).

For all models, follow-up bootstrapped analyses of partial correlations tested if the pattern of neurodegeneration associated with each CSF biomarker was more strongly related to one signature versus the other. The threshold for statistical significance was set *a priori* at $p<0.05$. For all significant models, sensitivity analyses were performed repeating models excluding participants with predictor or outcome values >4 standard deviations from the group mean. Analyses were conducted using R version 3.5.2 (www.r-project.org).

3. Results

3.1. Participant Characteristics

Participants included 153 adults age 60 to 92 (72±6 years, 67% male, 93% non-Hispanic White, 33% *APOE*-ε4 carriers). CSF Aβ concentrations ranged 289 to 1195 pg/mL, p-tau concentrations ranged 13 to 157 pg/mL, t-tau concentrations ranged 77 to 1542 pg/mL, neurogranin concentrations ranged 57 to 462 pg/mL, and NFL concentrations ranged 268 to 4025 pg/mL. The Schwarz AD signature ranged 1.93 to 2.65 and the McEvoy AD signature ranged -9.71 to 6.53. See **Table 1** for participant characteristics for the entire sample and stratified by diagnosis. See **Supplemental Table 1** for participants characteristics stratified by sex.

3.2. CSF Biomarkers & the AD Signatures

Among all participants, higher CSF t-tau (β=-0.0001, p=0.005) and NFL concentrations (β=-0.0001, p=0.001) were associated with smaller Schwarz AD signature values only. Higher CSF p-tau was associated with smaller McEvoy AD signature values (β=-0.0163, p=0.04) and with smaller Schwarz AD signature values (β=-0.0008, p=0.06) but the latter observation did not meet the *a priori* significance threshold. Neither CSF Aβ nor CSF neurogranin were associated with either AD signature (p-values>0.14). See **Table 2** and **Figure 2** for details. Sensitivity models excluding outliers yielded similar results (data not shown). In bootstrapped analyses, the variance explained by CSF p-tau (95% confidence interval (CI) -0.04, 0.02), t-tau (95% CI -0.05, 0.03), and NFL (95% CI -0.08, 0.02) on the Schwarz signature was not statistically difference from the variance explained on the McEvoy signature for each

biomarker. See **Table 3** for details.

3.3. CSF Biomarkers as Competing Predictors of Atrophy in AD Signature Regions

To determine whether the CSF biomarkers represent a common pathological pathway to atrophy in AD signature regions, a linear regression model was fit including all statistically significant CSF biomarker predictors for each signature. For the Schwarz AD signature, higher CSF NFL remained associated with lower Schwarz signature values (β=-0.00005, p=0.01), while the association with CSF t-tau was slightly attenuated (β=-0.0001, p=0.09). See **Table 4** for details. To compare the effect size of associations across CSF biomarkers, a change in R^2 was calculated for the competing models. The addition of CSF NFL to the model contributed 5.6% of variance beyond covariates and the addition of CSF t-tau contributed 3.5% of variance beyond covariates. Together, CSF NFL and t-tau contributed 6.6% of variance beyond covariates.

For the McEvoy AD signature, higher CSF NFL (β=-0.0011, p=0.005) remained associated with lower McEvoy signature values in a combined model with t-tau. Similarly, when CSF NFL was combined with p-tau, only NFL remained associated with McEvoy signature values (β=-0.0012, p<0.002). See **Table 4** for details. To compare the effect size of associations across CSF biomarkers, a change in R^2 was calculated for the competing models. The addition of CSF NFL to the model contributed 8.5% of variance beyond covariates, the addition of CSF t-tau contributed 4.5% of variance beyond covariates, and the addition of CSF p-tau contributed 1.6% of variance beyond covariates. Together, CSF NFL, t-tau and p-tau contributed 14.6% of variance beyond

covariates.

3.4. CSF Biomarkers x Cognitive Diagnosis Interactions & the AD Signatures

CSF p-tau (p=0.002), t-tau (p=0.003), and neurogranin (p=0.03) interacted with cognitive diagnosis on the Schwarz AD signature. CSF Aβ and NFL did not interact with diagnosis (p-values>0.10). In stratified analyses, higher CSF p-tau (β=-0.0025, p=0.009), t-tau (β=-0.0002, p=0.002), and NFL (β=-0.0001, p=0.001) were associated with smaller Schwarz AD signature values among MCI participants only. CSF Aβ (p=0.04), p-tau (p=0.002), t-tau (p=0.001), and neurogranin (p=0.03) interacted with cognitive diagnosis on the McEvoy AD signature but NFL did not (p=0.14). Similarly, stratified analyses revealed that higher CSF p-tau (β=-0.0303, p=0.02), t-tau (β=-0.0040, p=0.004), and NFL (β=-0.0016, p=0.003) were associated with smaller McEvoy AD signature values among MCI participants only. See **Table 5** for details. Sensitivity models excluding outliers yielded similar results (data not shown). In bootstrapped analyses, interactions and stratified associations did not differ in variance explained between the two signatures. See **Table 3** for details.

3.5. CSF Biomarkers x Sex Interactions & the AD Signatures

CSF p-tau (p=0.05) and neurogranin (p=0.04) interacted with sex on the Schwarz AD signature. Stratified models revealed that higher CSF p-tau (β=-0.0015, p=0.04) and t-tau (β=-0.0001, p=0.01) related to lower Schwarz AD signature values in females only, while the association with neurogranin approached significance (β=-0.0005, p=0.06). CSF neurogranin interacted with sex on the McEvoy AD signature (p=0.04), such that

associations were present in females only (β=-0.0073, p=0.04). Stratified analyses also revealed that higher CSF t-tau (β=-0.0027, p=0.02) related to lower McEvoy AD signature values in females only. CSF Aβ and NFL did not interact with sex on either AD signature (p-values>0.07). See **Table 6** and **Figure 3** for details. Sensitivity models excluding outliers yielded similar results (data not shown). In bootstrapped analyses, interactions and stratified associations did not differ in variance explained between the two signatures. See **Table 3** for details.

3.6. CSF Biomarkers x APOE-ε4 Carrier Status Interactions & the AD Signatures

No CSF biomarker interacted with *APOE*-ε4 status on either AD signature (p-values>0.11). In models stratified by *APOE*-ε4 status, higher CSF t-tau (β=-0.0001, p=0.02) and NFL (β=-0.0001, p=0.006) related to smaller Schwarz AD signature values among *APOE*-ε4 non-carriers. CSF NFL (β=-0.0014, p<0.0001) also related to smaller McEvoy AD signature values among *APOE*-ε4 non-carriers. See **Table 7** for details. Sensitivity models excluding outliers yielded similar results (data not shown). In bootstrapped analyses, stratified associations did not differ in variance explained between the two signatures. See **Table 3** for details.

3.7. CSF Biomarkers x Age Interactions & the AD Signatures

No CSF biomarker interacted with age on either AD signature (p-values>0.11). However, stratified analyses revealed that higher CSF t-tau (β=-0.0001, p=0.04) and NFL (β=-0.0001, p=0.02) related to lower Schwarz AD signature values among individuals over age 73. Similarly, higher CSF t-tau (β=-0.0030, p=0.02) and NFL (β=-

0.0014, p=0.001) related to lower McEvoy AD signature values among individuals over age 73. See **Table 8** for details. Sensitivity models excluding outliers yielded similar results (data not shown). In bootstrapped analyses, stratified associations did not differ in variance explained between the two signatures. See **Table 3** for details.

4. Discussion

Among community-dwelling older adults, higher concentrations of CSF p-tau, t-tau, and NFL were associated with lower AD signature values, indicating greater atrophy in grey matter regions affected by AD pathology. As expected, cognitive diagnosis modified the associations between CSF p-tau, t-tau, and neurogranin and AD signature values, such that associations were stronger among participants with MCI. Sex also modified associations between CSF p-tau and neurogranin, such that associations were stronger in females. Collectively, results suggest that CSF biomarkers of p-tau, t-tau, synaptic dysfunction, and axonal injury are associated with AD-related atrophy measured on MRI, and p-tau and synaptic dysfunction may be more prominent in AD-specific regions in females.

This study is among the first to show associations linking *in vivo* molecular biomarkers of core AD and concomitant pathologies with atrophy in AD-specific regions. As expected, higher levels of CSF p-tau and t-tau are associated with atrophy in the AD signatures among all participants, extending prior work focusing only on individuals with MCI.[6,9] As tau becomes more phosphorylated, it is no longer able to bind to microtubules within the axon,[22] compromising its ability to maintain cell structure and leading to cell death.[23] Additionally, it is well established that tau tangles first develop in anatomical regions included in the AD signatures[24] and *in vivo* atrophy in the Schwarz AD signature is associated with greater tangle deposition at autopsy.[6] Thus, increased levels of CSF p-tau and t-tau may indicate clinically relevant atrophy in AD-specific regions. Despite some previous work demonstrating Aβ relates to cortical thinning in AD signature regions,[8] we did not detect an association between CSF Aβ and AD-related

atrophy. It is notable this prior study included individuals with clinical AD, suggesting Aβ may not contribute to neurodegeneration in these regions until later disease states.

We also found an association between CSF NFL, a biomarker of axonal injury,[25] and AD-related atrophy. Importantly, CSF NFL explained variance in the AD signatures beyond that of CSF p-tau or t-tau, suggesting that axonal injury uniquely contributes to atrophy in these regions. NFL is a support protein within large axons[25] that is released into the CSF in a variety of pathological states, including vascular disease,[26] inflammation,[27] and neurodegeneration.[11] Regions included in the AD signatures, such as the entorhinal cortex and hippocampus, are vulnerable to ischemic small vessel disease[28] and chronic inflammation,[29,30] possibly contributing to axonopathy in these regions. Though increased CSF NFL is not specific to AD, axonal injury does contribute to AD-related atrophy, highlighting the importance of pathologies other than Aβ and tau in the development of AD-related atrophy and axonal injury as a potential therapeutic target in AD.

Results suggest that diagnosis modifies CSF p-tau, t-tau, and neurogranin associations with the AD signatures, such that associations are only present in participants with MCI. Additionally, though a formal interaction was not detected, CSF NFL was associated with the AD signatures only among participants with MCI. These findings are consistent with prior work showing that CSF p-tau, t-tau,[31] NFL,[11] and neurogranin[32] are elevated in individuals with MCI, likely leading to greater MRI evidence of neurodegeneration in AD-specific regions. Notably, though neurogranin was not associated with either AD signature among the entire sample, the diagnostic interaction and stratified results suggest that increased CSF neurogranin has a greater

contribution to AD-related atrophy in MCI compared to NC participants. Neurogranin is a post-synaptic protein prominent in the hippocampus[33] critical for long-term potentiation and synaptic plasticity.[34] While neurogranin tissue levels are decreased in AD,[35] CSF levels rise, presumably leaking into the CSF as synapses become disrupted and misfire. As synaptic dysfunction begins prior to overt tau tangle formation,[32] apoptotic cascades are initiated,[36] followed by neurodegeneration and cognitive impairment.[37] Given the importance and relative overexpression of neurogranin in regions vulnerable to AD pathology,[35,38] synaptic dysfunction in MCI, indicated by increased CSF neurogranin, may be an etiology underlying AD-related atrophy. CSF neurogranin is also elevated in conditions other than AD, such as stroke,[39] suggesting synaptic dysfunction may serve as one pathway by which vascular disease directly contributes to AD-related atrophy.

We found that sex modifies CSF p-tau and neurogranin associations with the AD signatures, such that associations are only present in females. While there is not a significant interaction, stratified results suggest that associations between CSF t-tau and the AD signatures are also female specific. This observation is consistent with prior work showing that the association between CSF t-tau and hippocampal volume is stronger in females.[13] Though atrophy in AD signature regions distinguishes NC from AD in both males and females,[6,7] our results suggest that the underlying pathology may differ. Estrogen is protective of tau hyperphosphorylation[40] and modulates dendritic spine density in the hippocampus,[41,42] so the decline in estrogen in aging females may lead to increased p-tau, synaptic dysfunction, and subsequent neurodegeneration. Additionally, women generally have fewer synapses in the temporal lobe,[43] suggesting that a given degree of synaptic dysfunction in aging in the temporal lobe may exert a

much greater effect on neuronal health in women compared to men. Thus, p-tau and synaptic dysfunction may be more prominent etiologies of AD-related atrophy in women, adding to the growing body of literature showing sex differences in pathology and structural brain changes in AD.[13,44] Interestingly, CSF NFL did not interact with sex on either AD signature, despite men having higher concentrations. It is possible that the effect of axonal injury on atrophy is more robust than other pathologies,[37] such that any sex-specific drivers of axonal injury are not strong enough to alter the association with atrophy. Future work is needed to better understand the mechanisms underlying sex-specific associations reported here and elsewhere.[13,44,45]

APOE-ε4 carrier status did not modify associations between CSF biomarkers and the AD signatures. Prior work suggests that the effects of *APOE*-ε4 on brain health are modified by sex and biomarker status.[13,45] Thus, it is possible the effects of *APOE*-ε4 on AD-related atrophy are also modified by these variables. Future work is needed with replication in larger samples to better understand these complicated three-way interactions and elucidate mechanisms by which *APOE*-ε4 affects neurodegeneration. Additionally, age did not modify any of the associations reported here, likely because the AD signature regions were originally selected based on their ability to separate age-related neurodegeneration from AD-specific neurodegeneration.[6,7]

Finally, one observation worth noting is that none of the associations reported here were significantly different between the Schwarz[6] and McEvoy[7] AD signatures. Despite the McEvoy signature including hippocampal volume and cortical thickness measurements that differ from the brain regions in the Schwarz signature, the two measures do not provide different information regarding the CSF biomarkers assessed

here. That is, one signature is not more sensitive to detecting atrophy associated with AD pathology, neurodegeneration, synaptic dysfunction, or axonopathy compared to the other. Future research is needed to further delineate differences in the two signatures (and others not examined here)[46-48] and determine if specific regions within the signatures are more or less vulnerable to certain pathologies.

The current study has several strengths, including a clinically well characterized cohort with excellent methods for quantifying cortical thickness and CSF biomarkers of AD and concomitant pathology. Additional strengths include comprehensive ascertainment of potential confounders, a comprehensive investigation of concomitant pathways to neurodegeneration beyond primary AD pathology, and the use of core laboratories using quality control procedures to analyze all CSF and brain MRI measurements in batch with technicians blinded to participant clinical information. However, limitations include the cross-sectional methods, which cannot address causality. Longitudinal studies are needed to understand how AD pathology, synaptic dysfunction, and axonal injury affect AD-related neurodegeneration over time. Additionally, the cohort was older and predominantly non-Hispanic White, thus limiting generalizability to other races, ethnicities, and age groups.

This study demonstrates novel associations linking CSF biomarkers of neurodegeneration and axonopathy with atrophy in regions vulnerable to AD-related neurodegeneration. Additionally, sex modified associations, such that p-tau, t-tau, and neurogranin were associated with AD-related atrophy in females only. These findings suggest that axonal injury driving AD-related neurodegeneration and neuronal dysfunction may be more prominent in these regions in females. Future research is

needed to better understand how these pathologies regionally interact to precipitate cognitive decline and sex-specific etiologies of neurodegeneration.

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Figure 1. Participant Inclusion/Exclusion Details

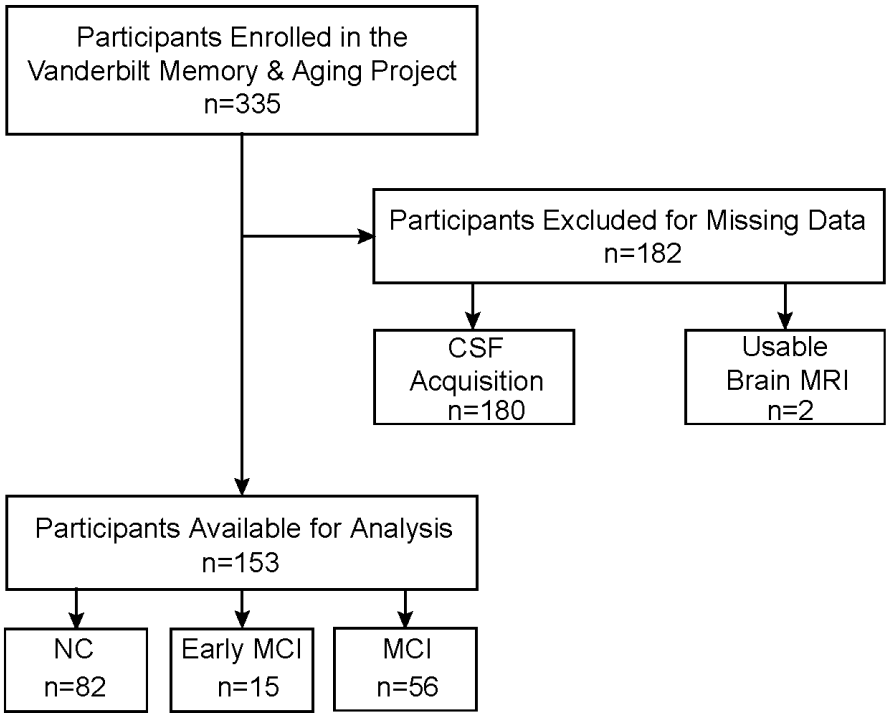
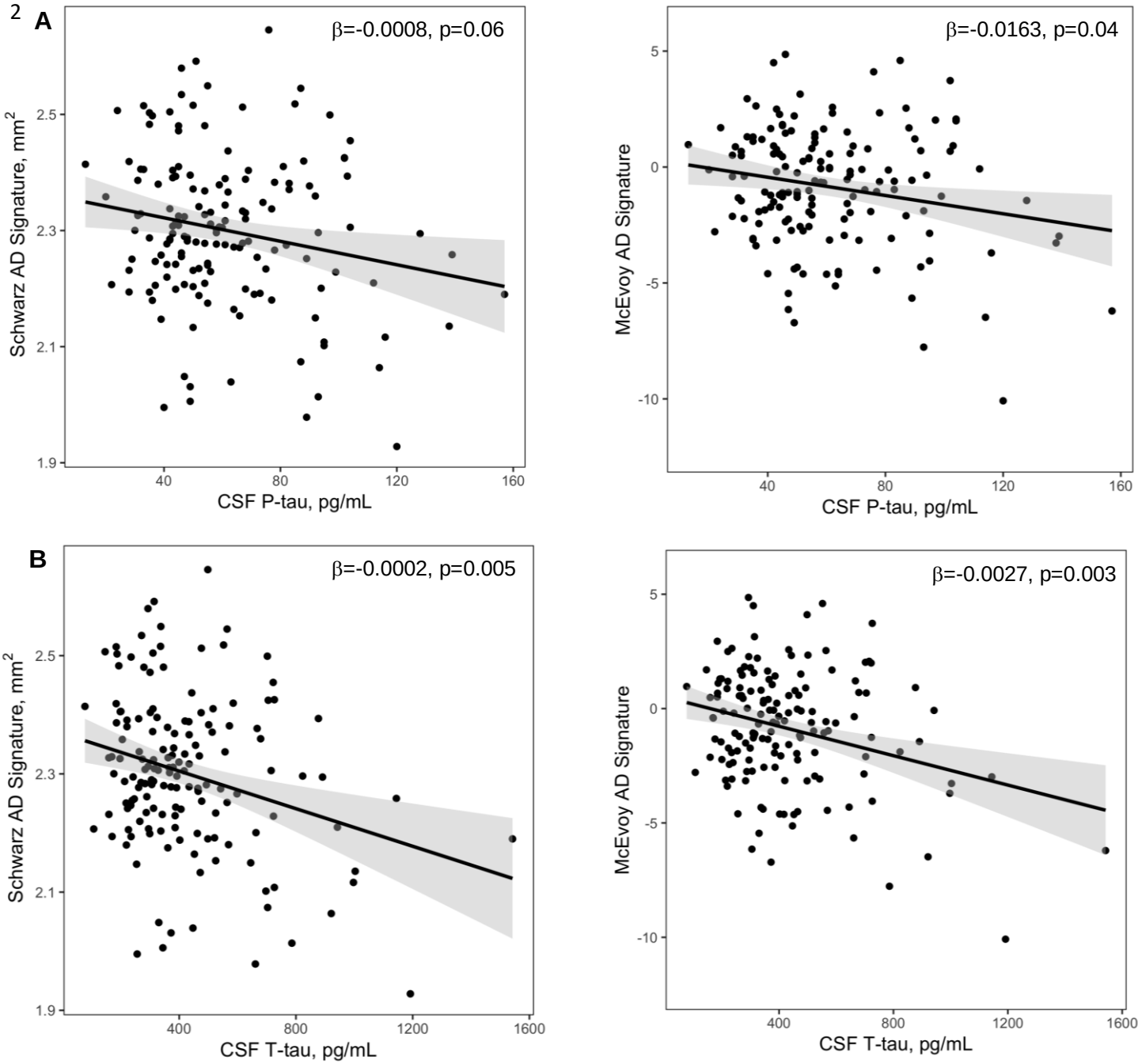


Figure 1. Missing data categories are mutually exclusive. An additional 5 participants were excluded from analyses examining CSF neurofilament light. CSF=cerebrospinal fluid; MCI=mild cognitive impairment; MRI=magnetic resonance imaging; NC=normal cognition.

Figure 2. CSF Biomarker Associations with the AD Signature



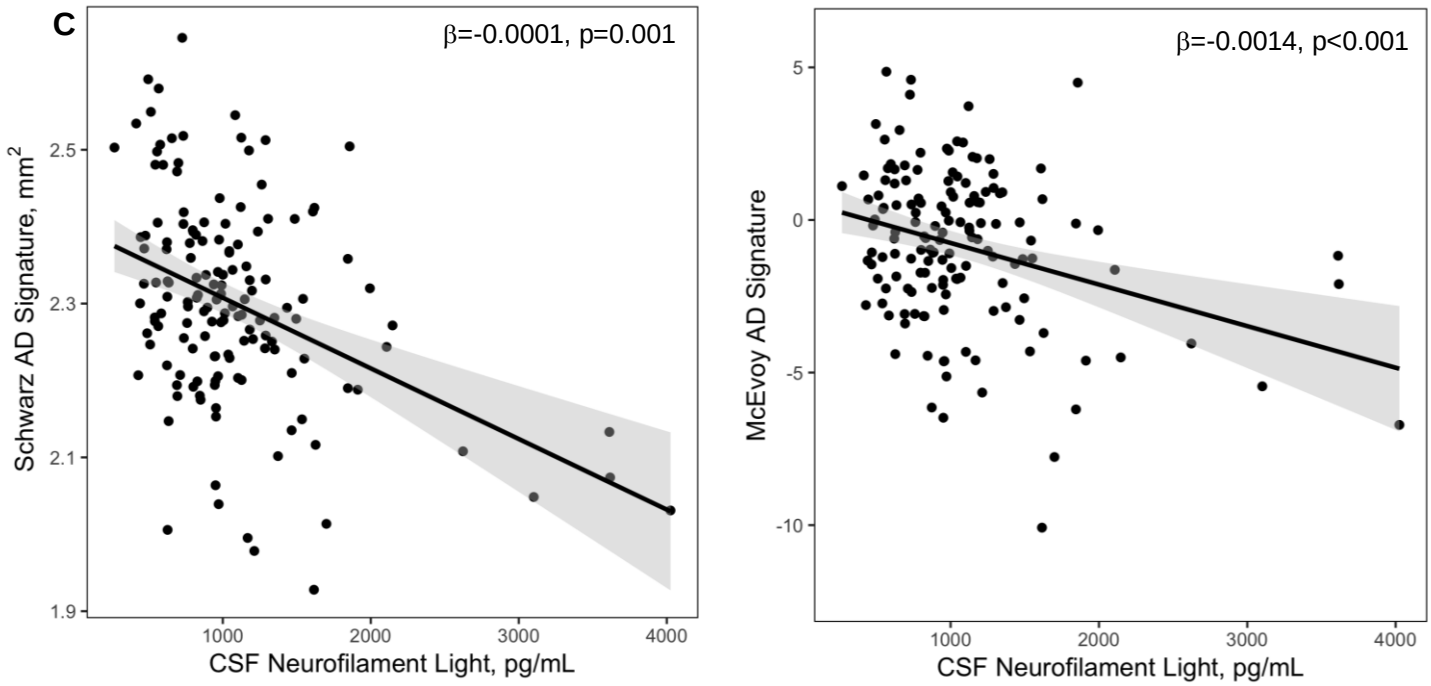


Figure 2. Solid lines reflect values of the AD signature corresponding to CSF biomarkers. Shading reflects 95% confidence interval. **A)** Associations between CSF p-tau and the AD signatures; **B)** Associations between CSF t-tau and the AD signatures; **C)** Associations between CSF NFL and the AD signatures. AD=Alzheimer's disease; CSF=cerebrospinal fluid; NFL=neurofilament light; P-tau=phosphorylated tau; T-tau=total tau.

Figure 3. CSF Biomarker x Sex Interactions with the AD Signatures

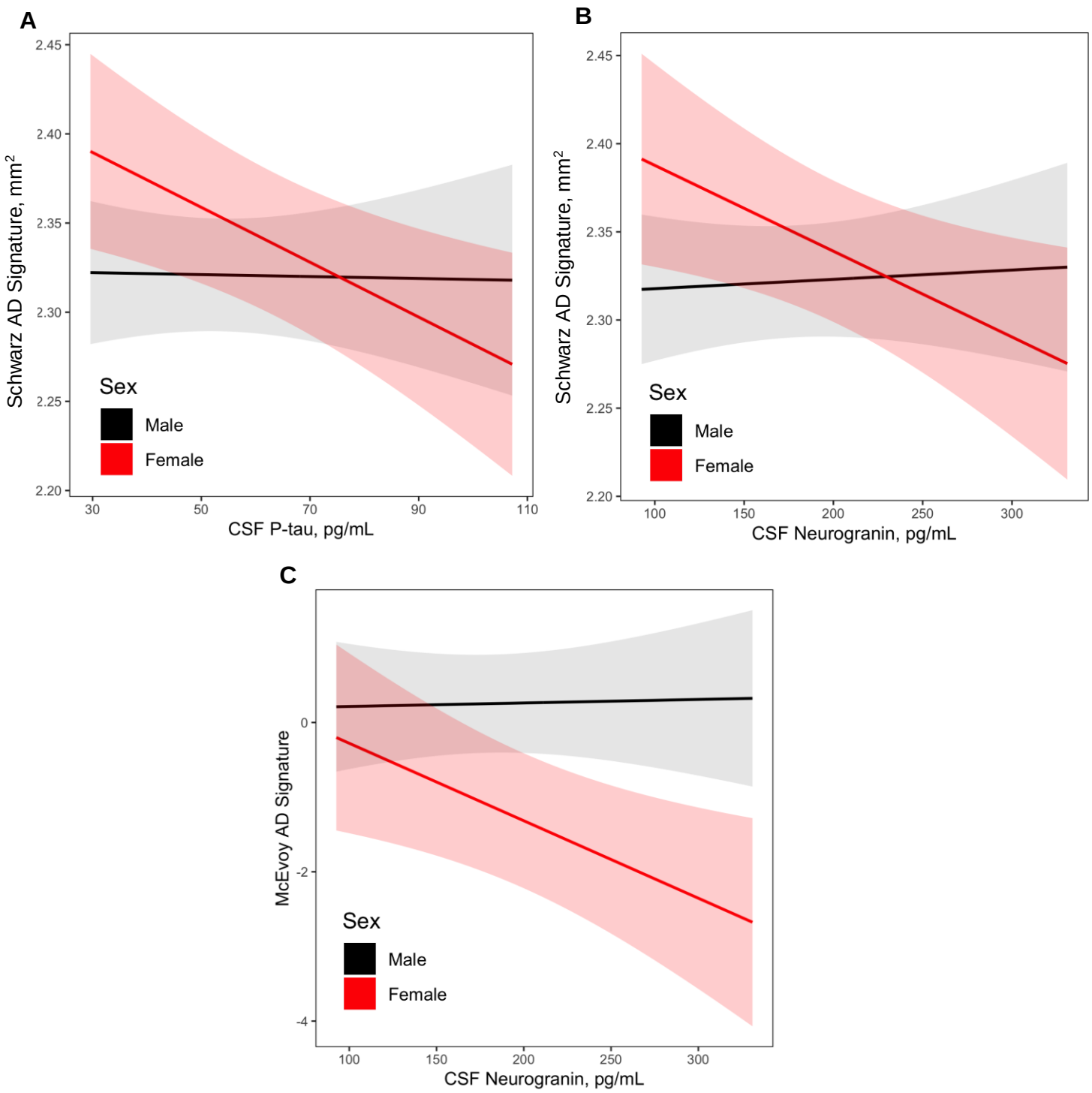


Figure 3. Lines reflect values of the AD signatures corresponding to CSF biomarkers. Shading reflects 95% confidence interval. **A)** Associations between CSF p-tau and the Schwarz AD signature, stratified by sex; male β=-0.0004, p=0.44; female β=-0.0011, p=0.04; **B)** Associations between CSF neurogranin and the Schwarz AD signature, stratified by sex; male β=-0.00002, p=0.91; female β=-0.0004, p=0.06; **C)** Associations between CSF neurogranin and the McEvoy AD signature, stratified by sex; male β=-0.0009, p=0.78; female β=-0.0073; p=0.04; AD=Alzheimer's disease; CSF=cerebrospinal fluid; P-tau=phosphorylated tau.

Table 1. Participant Characteristics

Demographic Characteristics	Total n=153	NC n=82	eMCI n=15	MCI n=56	p-value
Age, years	72±6	72±7	73±6	73±6	0.90
Sex, % male	67	70	80	61	0.30
Race, % Non-Hispanic White	93	94	93	91	0.82
Education, years	16±3	17±2	16±3	15±3	**0.002***
APOE-ε4, % carrier	33	28	13	45	**0.03**
Schwarz AD signature, mm^2	2.3±0.13	2.3±0.12	2.3±0.09	2.2±0.14	**<0.001*†**
McEvoy AD signature	-0.85±2.5	-0.09±2.1	-0.87±2.2	-1.96±2.9	**<0.001***
CSF Biomarkers, pg/mL					
Aβ	718±245	765±226	817±282	622±234	**<0.001*†**
P-tau	61±26	56±22	63±17	67±31	0.06
T-tau	425±227	373±175	429±125	500±290	**0.02***
Neurogranin	196±77	185±74	213±73	206±82	0.21
Neurofilament Light	1070±582	939±453	1088±465	1250±718	**0.004***

Note. Values denoted as mean±standard deviation or frequency. P-values were generated using a Kruskal-Wallis test for continuous variables and a Pearson test for categorical variables. *NC is different than MCI. †eMCI is different than MCI. Aβ=amyloid beta; AD=Alzheimer's disease; APOE=apolipoprotein E; CSF=cerebrospinal fluid; eMCI=early MCI; MCI=mild cognitive impairment; NC=normal cognition; P-tau=phosphorylated tau; T-tau=total tau.

Table 2. CSF Biomarker Associations with the AD Signatures

	β	95% Confidence Intervals	p-value
Schwarz AD Signature			
Aβ	0.00004	-0.00005, 0.0001	0.36
P-tau	-0.0008	-0.0015, 0.00003	0.06
T-tau	-0.0001	-0.0002, -0.00004	**0.005**
Neurogranin	-0.0002	-0.0004, 0.0001	0.23
NFL	-0.00006	-0.0001, -0.00002	**0.001**
McEvoy AD Signature			
Aβ	0.0009	-0.0010, 0.0029	0.39
P-tau	-0.0163	-0.0319, -0.0007	**0.04**
T-tau	-0.0027	-0.0045, -0.0009	**0.003**
Neurogranin	-0.0036	-0.0082, 0.0011	0.14
NFL	-0.0014	-0.0021, -0.0007	**<0.001**

Note. Models were adjusted for age, sex, education, race/ethnicity, diagnosis, and *APOE*-ε4 status, Models examining the McEvoy AD signature were also covaried for intracranial volume. An additional 5 participants were excluded from the CSF NFL analyses. β indicates the degree of change in volume of AD signature per 1 pg/mL increase in each CSF biomarker. Aβ= amyloid beta; AD=Alzheimer's Disease; *APOE*=apolipoprotein E; NFL=neurofilament light; P-tau=phosphorylated tau; T-tau=total tau.

Table 3. Statistical Comparison Between CSF Biomarkers Associations with the AD Signatures

	Schwarz Incremental R^2	McEvoy Incremental R^2	Difference in Incremental R^2	95% Confidence Intervals
Main Effects				
P-tau	0.019	0.023	-0.005	-0.038, 0.019
T-tau	0.041	0.048	-0.007	-0.050, 0.030
NFL	0.057	0.084	-0.027	-0.078, 0.017
Diagnostic Interactions				
Aβ	0.016	0.035	-0.019	-0.071, 0.010
P-tau	0.053	0.078	-0.025	-0.085, 0.016
T-tau	0.048	0.079	-0.031	-0.093, 0.009
Neurogranin	0.029	0.050	-0.021	-0.084, 0.011
MCI Participants Only				
P-tau	0.103	0.088	0.015	-0.065, 0.083
T-tau	0.134	0.131	0.004	-0.064, 0.078
NFL	0.156	0.139	0.017	-0.079, 0.104
Sex Interactions				
P-tau	0.020	0.047	-0.027	-0.095, 0.007
Neurogranin	0.022	0.056	-0.033	-0.108, 0.005
Female Participants Only				
P-tau	0.050	0.048	0.003	-0.076, 0.056
T-tau	0.067	0.070	-0.003	-0.082, 0.065
Neurogranin	0.040	0.053	-0.012	-0.076, 0.042
NFL	0.063	0.082	-0.019	-0.092, 0.046
Male Participants Only				
NFL	0.068	0.100	-0.032	-0.101, 0.034
***APOE*-ε4 Non-Carriers Only**				
T-tau	0.043	0.025	0.018	-0.017, 0.071
NFL	0.059	0.102	0.231	-0.121, 0.015
Participants >73 Only				
T-tau	0.046	0.058	-0.020	-0.055, 0.057
NFL	0.062	0.109	-0.047	-0.121, 0.035

Note. Confidence intervals crossing 0 indicate that there is not a statistically significant difference in the variance explained by the predictor on the two signatures. Difference in incremental R^2 calculated as Schwarz incremental R^2 – McEvoy Incremental R^2. Aβ= amyloid beta; *APOE*=apolipoprotein E; NFL=neurofilament light; P-tau=phosphorylated tau; T-tau=total tau.

Table 4. Combined Models of CSF Biomarkers in Relation to the AD Signatures

	β	95% Confidence Intervals	p-value
Schwarz AD Signature (T-tau + NFL)			
T-tau	-0.0001	-0.0002, 0.00001	0.09
NFL	-0.00005	-0.0001, -0.00001	**0.01**
McEvoy AD Signature (T-tau + NFL)			
T-tau	-0.002	-0.0036, 0.0003	0.09
NFL	-0.0011	-0.0018, -0.0003	**0.005**
McEvoy AD Signature (P-tau + NFL)			
P-tau	-0.007	-0.0231, 0.0096	0.42
NFL	-0.0012	-0.0020, -0.0005	**0.002**

Note. Analyses performed on n=148. Combined models only included biomarkers significantly associated with each signature when analyzed individually (**Table 2**). Models were adjusted for age, sex, education, race/ethnicity, diagnosis, and *APOE*-ε4 status, Models examining the McEvoy AD signature were also covaried for intracranial volume. β indicates the degree of change in volume of AD signature per 1 pg/mL increase in each CSF biomarker. AD=Alzheimer's Disease; *APOE*=apolipoprotein E; CSF=cerebrospinal fluid; NFL=neurofilament light; P-tau=phosphorylated tau; T-tau=total tau.

Table 5. CSF Biomarker x Diagnosis Interactions on the AD Signatures

	CSF Biomarker x Diagnosis* Interaction n=138			**NC n=82†**			**MCI n=56†**		
Schwarz AD Signature	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	**p-value**	**β**	**95% CI**	***p*-value**
Aβ	0.0002	-0.00003, 0.0003	0.10	0.00004	-0.0001, 0.0002	0.53	0.0001	-0.0001, 0.0003	0.47
P-tau	-0.0025	-0.0041, -0.0009	**0.002**	0.0004	-0.0008, 0.0016	0.51	-0.0016	-0.0027, -0.0004	**0.009**
T-tau	-0.0003	-0.0005, 0.0001	**0.003**	0.00003	-0.0001, 0.0002	0.73	-0.0002	-0.0003, 0.0001	**0.002**
Neurogranin	-0.0006	-0.0011, -0.0001	**0.03**	0.0001	-0.0003, 0.0004	0.71	-0.0003	-0.0008, 0.0001	0.16
NFL	-0.0001	-0.0001, 0.00001	0.11	-0.00001	-0.0001, 0.0001	0.74	-0.0001	-0.0001, -0.00003	**0.001**
	CSF Biomarker x Diagnosis* Interaction n=138			**NC n=82†**			**MCI n=56†**		
McEvoy AD Signature	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**
Aβ	0.0039	0.0003, 0.0076	**0.04**	0.0009	-0.0016, 0.0033	0.48	0.0027	-0.0017, 0.0072	0.22
P-tau	-0.0493	-0.0802, -0.0802	**0.002**	0.0077	-0.0142, 0.0296	0.49	-0.0303	-0.0555, -0.0052	**0.02**
T-tau	-0.006	-0.0096, -0.0024	**0.001**	0.0008	-0.0020, 0.0036	0.58	-0.0040	-0.0066, -0.0014	**0.004**
Neurogranin	-0.0118	-0.0224, -0.0012	**0.03**	0.0010	-0.0054, 0.0074	0.76	-0.0066	-0.0170, 0.0038	0.21
NFL	-0.0011	-0.0026, 0.0004	0.14	-0.0004	-0.0017, 0.0008	0.51	-0.0016	-0.0026, -0.0006	**0.003**

Note: *Models were adjusted for age, sex, education, race/ethnicity, diagnosis, and *APOE*-ε4 status. †Models were adjusted for age, sex, education, race/ethnicity, and *APOE*-ε4 status. Models examining the McEvoy AD signature were also covaried for intracranial volume. An additional 5 participants were excluded from the CSF NFL analyses. β indicates the degree of change in volume per 1 pg/mL increase in each CSF biomarkers. Aβ=amyloid beta; AD=Alzheimer's disease; *APOE*=apolipoprotein E; CI=confidence interval; CSF=cerebrospinal fluid; MCI=mild cognitive impairment; NC=normal cognition; NFL=neurofilament light; P-tau=phosphorylated tau; T-tau=total tau.

Table 6. CSF Biomarker x Sex Interactions on the AD Signatures

	CSF Biomarker x Sex* Interaction n=153			**Female n=50†**			**Male n=103†**		
Schwarz AD Signature	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	**p-value**	**β**	**95% CI**	***p*-value**
Aβ	0.0001	-0.00005, 0.0003	0.14	0.00003	-0.0001, 0.0002	0.73	0.00007	-0.00005, 0.0002	0.26
P-tau	-0.0015	-0.0030, -0.000001	**0.05**	-0.0011	-0.0022, -0.0001	**0.04**	-0.0004	-0.0015, 0.0007	0.44
T-tau	-0.0001	-0.0003, 0.00002	0.08	-0.0001	-0.0003, -0.00003	**0.01**	-0.0001	-0.0002, 0.00004	0.16
Neurogranin	-0.0005	-0.0011, -0.00003	**0.04**	-0.0004	-0.0008, 0.00002	0.06	-0.00002	-0.0004, 0.0003	0.91
NFL	-0.00004	-0.0001, 0.00003	0.26	-0.0001	-0.0001, -0.00001	**0.02**	-0.0001	-0.0001, -0.00002	**0.005**
	CSF Biomarker x Sex* Interaction n=153			**Female n=50)†**			**Male n=103†**		
McEvoy AD Signature	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**
Aβ	0.0032	-0.0003, 0.0068	0.07	0.0001	-0.0042, 0.0044	0.96	0.0014	-0.0011, 0.0038	0.27
P-tau	-0.0246	-0.0543, 0.0051	0.10	-0.0201	-0.0411, 0.0008	0.06	-0.0112	-0.0332, 0.0108	0.31
T-tau	-0.0026	-0.0060, 0.0008	0.13	-0.0027	-0.0049, -0.0004	**0.02**	-0.0021	-0.0048, 0.0006	0.12
Neurogranin	-0.0098	-0.0190, -0.0006	**0.04**	-0.0073	-0.0144, -0.0002	**0.04**	-0.0009	-0.0069, 0.0052	0.78
NFL	-0.0006	-0.0021, 0.0021	0.39	-0.0016	-0.0028, -0.0003	**0.01**	-0.0015	-0.0024, 0.0006	**0.001**

Note: *Models were adjusted for age, sex, education, race/ethnicity, diagnosis, and *APOE*-ε4 status. †Models were adjusted for age, education, race/ethnicity, diagnosis, and *APOE*-ε4 status. Models examining the McEvoy AD signature were also covaried for intracranial volume. An additional 5 participants were excluded from the CSF NFL analyses. β indicates the degree of change in volume per 1 pg/mL increase in each CSF biomarkers. Aβ=amyloid beta; AD=Alzheimer's disease; *APOE*=apolipoprotein E; CI=confidence interval; CSF=cerebrospinal fluid; MCI=mild cognitive impairment; NC=normal cognition; NFL=neurofilament light; P-tau=phosphorylated tau; T-tau=total tau.

Table 7. CSF Biomarker x *APOE*-ε4 Carrier Status Interactions on the AD Signatures

	CSF Biomarker x APOE-ε4* Interaction n=153			***APOE*-ε4 Carrier n=50†**			***APOE*-ε4 Non-Carrier n=103†**		
Schwarz AD Signature	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	**p-value**	**β**	**95% CI**	***p*-value**
Aβ	0.0001	-0.0001, 0.0003	0.29	0.0001	-0.0001, 0.0003	0.46	0.00004	-0.0001, 0.0001	0.53
P-tau	0.0001	-0.0015, 0.0017	0.87	-0.0001	-0.0015, 0.0013	0.88	-0.0009	-0.0019, 0.0001	0.08
T-tau	0.00001	-0.0002, 0.0002	0.96	-0.0001	-0.0002, 0.0001	0.50	-0.0001	-0.0003, -0.00002	**0.02**
Neurogranin	-0.0001	-0.0006, 0.0005	0.86	0.00004	-0.0005, 0.0006	0.89	-0.0002	-0.0005, 0.0001	0.32
NFL	0.00001	-0.0001, -0.0001	0.91	-0.0001	-0.0001, 0.00003	0.19	-0.0001	-0.0001, -0.00002	**0.006**
	CSF Biomarker x APOE-ε4* Interaction n=153			***APOE*-ε4 Carrier n=50†**			***APOE*-ε4 Non-Carrier n=103†**		
McEvoy AD Signature	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**
Aβ	0.0033	-0.0008, 0.0073	0.11	0.0029	-0.0009, 0.0067	0.13	-0.00004	-0.0024, 0.0023	0.97
P-tau	-0.0126	-0.0448, 0.0195	0.44	-0.0065	-0.0329, 0.0198	0.62	-0.0124	-0.0324, 0.0076	0.22
T-tau	-0.0018	-0.0053, 0.0018	0.33	-0.0015	-0.0044, 0.0014	0.30	-0.0020	-0.0044, 0.0003	0.09
Neurogranin	-0.0046	-0.0161, 0.0069	0.43	-0.0004	-0.0103, 0.0094	0.93	-0.0028	-0.0088, 0.0033	0.37
NFL	0.0001	-0.0014, 0.0016	0.94	-0.0008	-0.0023, 0.0007	0.30	-0.0014	-0.0022, -0.0006	**<0.001**

Note: *Models were adjusted for age, sex, education, race/ethnicity, diagnosis, and *APOE*-ε4 status. †Models were adjusted for age, sex, education, race/ethnicity, and diagnosis. Models examining the McEvoy AD signature were also covaried for intracranial volume. An additional 5 participants were excluded from the CSF NFL analyses. β indicates the degree of change in volume per 1 pg/mL increase in each CSF biomarkers. Aβ=amyloid beta; AD=Alzheimer's disease; *APOE*=apolipoprotein E; CI=confidence interval; CSF=cerebrospinal fluid; MCI=mild cognitive impairment; NC=normal cognition; NFL=neurofilament light; P-tau=phosphorylated tau; T-tau=total tau.

Table 8. CSF Biomarker x Age Interactions on the AD Signatures

	CSF Biomarker x Age* Interaction n=153			**<73 years old n=73†**			**≥73 years old n=80†**		
Schwarz AD Signature	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	**p-value**	**β**	**95% CI**	***p*-value**
Aβ	-0.0001	-0.0003, 0.00003	0.11	0.0001	-0.00001, 0.0003	0.07	-0.00002	-0.0002, 0.0001	0.73
P-tau	-0.0001	-0.0016, 0.0014	0.86	-0.0007	-0.0019, 0.0004	0.19	-0.0008	-0.0019, 0.0004	0.17
T-tau	-0.00004	-0.0002, 0.0001	0.66	-0.0001	-0.0002, 0.00001	0.07	-0.0001	-0.0003, -0.00004	**0.04**
Neurogranin	0.0001	-0.0004, 0.0006	0.74	-0.0002	-0.0006, 0.0001	0.22	-0.0001	-0.0005, 0.0003	0.74
NFL	0.00001	-0.0001, 0.0001	0.74	-0.0001	-0.0001, 0.00001	0.08	-0.0001	-0.00001, -0.00001	**0.02**
	CSF Biomarker x Age* Interaction n=153			**<73 years old n=73†**			**≥73 years old n=80†**		
McEvoy AD Signature	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**
Aβ	-0.0026	-0.0057, 0.0006	0.11	0.0024	-0.0010, 0.0058	0.16	-0.0001	-0.0028, 0.0025	0.92
P-tau	-0.0080	-0.0378, 0.0218	0.60	-0.0123	-0.0365, 0.0120	0.31	-0.0192	-0.0410, 0.0026	0.08
T-tau	-0.0011	-0.0044, 0.0023	0.52	-0.0022	-0.0049, 0.0006	0.12	-0.0030	-0.0055, -0.0005	**0.02**
Neurogranin	-0.0014	-0.0116, 0.0089	0.79	-0.0030	-0.0108, 0.0048	0.45	-0.0033	-0.0110, 0.0043	0.39
NFL	-0.0001	-0.0016, 0.0014	0.86	-0.0012	-0.0027, 0.0003	0.12	-0.0014	-0.0023, -0.0006	**0.001**

Note: *Models were adjusted for age, sex, education, race/ethnicity, diagnosis, and *APOE*-ε4 status. †Models were adjusted for sex, education, race/ethnicity, *APOE*-ε4 status, and diagnosis. Models examining the McEvoy AD signature were also covaried for intracranial volume. An additional 5 participants were excluded from the CSF NFL analyses. β indicates the degree of change in volume per 1 pg/mL increase in each CSF biomarkers. Aβ=amyloid beta; AD=Alzheimer's disease; *APOE*=apolipoprotein E; CI=confidence interval; CSF=cerebrospinal fluid; MCI=mild cognitive impairment; NC=normal cognition; NFL=neurofilament light; P-tau=phosphorylated tau; T-tau=total tau.

Supplemental Table 1. Participant Characteristics Stratified by Sex

Demographic Characteristics	Female n=50	Male n=103	p-value
Age, years	72±7	72±6	0.69
APOE-ε4, % carrier	32	33	1
Race, % Non-Hispanic White	85	94	0.55
Education, years	15±3	17±3	**<0.001**
MCI, %	42	33	0.30
Schwarz AD Signature, mm^2	2.3±0.14	2.3±0.13	0.82
McEvoy AD Signature	-1.63±2.7	-0.47±2.4	**0.02**
CSF Biomarkers, pg/mL			
Aβ	636±228	756±244	**0.005**
P-tau	65±30	59±23	0.22
T-tau	471±285	403±191	0.20
Neurogranin	203±83	190±74	0.20
NFL	975±549	1114±594	**0.05**

Note. Values denoted as mean±standard deviation or frequency. P-values were generated using a Kruskal-Wallis test for continuous variables and a Pearson test for categorical variables. AD=Alzheimer's disease; APOE=apolipoprotein E; CSF=cerebrospinal fluid; MCI=mild cognitive impairment; NFL=neurofilament light; P-tau=phosphorylated tau; T-tau=total tau.