Cerebrospinal fluid β-amyloid$_{42}$ and neurofilament light relate to white matter hyperintensities

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Abstract

White matter hyperintensities (WMHs) are associated with poorer brain health, but their pathophysiological substrates remain elusive. To better understand the mechanistic underpinnings of WMHs among older adults, this study examined *in vivo* cerebrospinal fluid biomarkers of β-amyloid$_{42}$ deposition (Aβ$_{42}$), hyperphosphorylated tau pathology (p-tau), neurodegeneration (total tau), and axonal injury (neurofilament light; NFL) in relation to log-transformed WMHs volume. Participants free of clinical stroke and dementia were drawn from the Vanderbilt Memory & Aging Project (n=148, 72±6 years). Linear regression models adjusted for age, sex, race/ethnicity, education, intracranial volume, modified Framingham Stroke Risk Profile (excluding points assigned for age), cognitive diagnosis, and *APOE*-ε4 carrier status. Aβ$_{42}$ (β=-0.001, p=0.003) and NFL (β=0.0003, p=0.02) concentrations related to WMHs, but neither p-tau nor total tau associations with WMHs reached statistical significance (p-values>0.21). In a combined model, NFL accounted for 3.2% of unique variance in WMHs and Aβ$_{42}$ accounted for an additional 4.3% beyond NFL, providing novel evidence of the co-occurrence of at least two distinct pathways for WMHs among older adults, including amyloidosis and axonal injury.

Keywords: cerebrospinal fluid, dementia, white matter hyperintensities, amyloid, β-amyloid$_{42}$, neurofilament light

1. Introduction

White matter hyperintensities (WMHs) are nonspecific radiographic markers of fluid accumulation that appear on T2-weighted MRI. Prevalent among community-dwelling older adults (Garde et al., 2000; Ylikoski et al., 1995), WMHs relate to an increased incidence of clinical dementia (Benedictus et al., 2015; Debette et al., 2010; Provenzano et al., 2013) and stroke (Debette et al., 2010). Cerebral small vessel disease is traditionally the most common etiology attributed to WMHs in older adults (Provenzano et al., 2013), but emerging evidence suggests multiple pathological pathways are likely (Gouw et al., 2011). Among older adults with prodromal and clinical Alzheimer's disease (AD), increased WMHs relate to *in vivo* amyloid aggregation as measured by PET (Grimmer et al., 2012; Kandel et al., 2016; Marnane et al., 2016; Provenzano et al., 2013) and cerebrospinal fluid (CSF) β-amyloid$_{42}$ (Aβ_{42}) (Marnane et al., 2016). Increased WMHs are found in autosomal-dominant AD prior to the onset of clinical symptoms, suggesting a link between WMHs and AD pathophysiology (Lee et al., 2016). Increased tau aggregation has also been linked to WMHs (Hertze et al., 2013; Marnane et al., 2016; Tosto et al., 2015), albeit less consistently than amyloid aggregation (Kester et al., 2014; van Westen et al., 2016). Collectively, these data suggest there may be an AD-specific pathway contributing to WMHs among older adults.

CSF biomarkers have become increasingly recognized for their diagnostic and prognostic utility in the study of AD and related dementias (Olsson et al., 2016). CSF comes in direct contact with the extracellular compartment of the brain. Therefore, analysis of CSF can provide *in vivo* information about biochemical changes associated

with pathophysiological processes and may enhance insight into the neural correlates of WMHs. $A\beta_{42}$ and hyperphosphorylated tau (p-tau) are well-established CSF biomarkers associated with AD while total tau (t-tau) is a well-established CSF biomarker of neurodegeneration (Blennow et al., 2010). Neurofilament light (NFL) is a non-disease-specific CSF biomarker for large-caliber axonal injury (Skillback et al., 2014). **Figure 1** provides an illustration of the pathophysiology of AD and neuronal injury associated with changes in concentrations for each of these four CSF biomarkers of interest (Aβ42, p-tau, t-tau, and NFL). Despite emerging evidence that reduced CSF $A\beta_{42}$ (Kester et al., 2014; Marnane et al., 2016) and increased CSF NFL (Bjerke et al., 2014; Jonsson et al., 2010) may correlate with higher WMHs volume among older adults, it is unknown whether the variance explained by these two biomarkers is unique or overlapping. Furthermore, p-tau and t-tau associations with WMHs warrant further investigation given inconsistent findings to date (Kester et al., 2014).

The current study relates *in vivo* CSF biomarkers of AD pathophysiology ($A\beta_{42}$, p-tau), neurodegeneration (t-tau), and axonal injury (NFL) to fluid attenuated inversion recovery (FLAIR)-assessed cerebral WMHs. We hypothesized that $A\beta_{42}$ and NFL would relate to WMHs among older adults. If WMHs reflect heterogeneous AD-specific and non-AD-specific pathophysiological substrates, then biomarkers of AD pathogenesis ($A\beta_{42}$) and non-AD-specific axonal damage (NFL) should account for separate variance in WMHs. Therefore, in secondary analyses, we hypothesized that $A\beta_{42}$ and NFL would account for unique variance in WMHs. Given the mixed literature regarding p-tau and t-tau associations with WMHs (Kester et al., 2014; Marnane et al., 2016), we hypothesized that p-tau and t-tau would have weak associations with WMHs. The

novelty of our work lies in our inclusion of competitive analytical models incorporating both $A\beta_{42}$ and NFL. These models assess whether each biomarker is associated with WMHs and whether the variance in WMHs accounted for by each biomarker is overlapping or independent of the other. This approach provides a more integrated understanding of the underlying CSF biochemical changes that occur in the context of WMHs among older adults. Our research also adds to emerging evidence linking CSF NFL to brain health outcomes among participant samples enriched for prodromal AD.

2. Materials and methods

2.1 Participants

The Vanderbilt Memory & Aging Project (Jefferson et al., 2016) is a longitudinal study investigating vascular health and brain aging in a cohort enriched for mild cognitive impairment (MCI). Cohort inclusion criteria required participants be age 60 years and older, speak English, have adequate auditory and visual acuity for testing, and have a reliable study partner. At enrollment, participants were excluded for MRI contraindication, history of neurological disease (e.g., dementia, multiple sclerosis), stroke, heart failure, major psychiatric illness (e.g., schizophrenia), head injury with loss of consciousness >5 minutes, and systemic or terminal illness (e.g., cancer) that could impact follow-up examination participation. Based on a detailed medical history and record review, clinical interview, and neuropsychological assessment, participants were labeled at baseline with normal cognition (NC), early MCI (Aisen et al., 2010), or MCI (Albert et al., 2011). At enrollment participants completed a comprehensive evaluation over 2 or 3 days, including (but not limited to) fasting blood draw, medical history and

medication review, physical examination, and multi-modal brain MRI. Participation in a fasting lumbar puncture was optional. Participants were excluded from the current study if they were missing CSF, covariate, or brain MRI data (see **Figure 2** 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 Analyses

At baseline, participants were invited to complete an optional fasting lumbar puncture procedure. CSF was collected with polypropylene syringes using a Sprotte 25-gauge spinal needle in an intervertebral lumbar space. Samples were immediately mixed and centrifuged, and supernatants were aliquoted in 0.5mL polypropylene tubes and stored at -80°C. Samples were analyzed in batch using commercially available enzyme-linked immunosorbent assays (Fujirebio, Ghent, Belgium) to determine the levels of $A\beta_{42}$ (INNOTEST® β-AMYLOID$_{(1-42)}$), p-tau* (INNOTEST® PHOSPHO-TAU$_{(181P)}$), and t-tau (INNOTEST® hTAU). P-tau was measured by tagging a tau phosphorylation site at amino acid Thr181. This form of phosphorylated tau appears most specific to AD and correlates with tangle pathology (Buerger et al., 2006; Seppala et al., 2012). NFL was measured using a commercially available enzyme-linked immunosorbent assay (Uman Diagnostics). Board-certified laboratory technicians processed data blinded to clinical information, as previously described (Palmqvist et al., 2014). Intra-assay coefficients of variation were <10 percent.

2.3 Brain MRI

Participants were scanned at the Vanderbilt University Institute of Imaging

Science on a 3T Philips Achieva system (Best, The Netherlands) with 8-channel SENSE receiver head coil (Pruessmann et al., 1999). T1-weighted (repetition time=8.9ms, echo time=4.6ms, spatial resolution=1x1x1mm^3) and T2-weighted FLAIR (repetition time=11000ms, echo time=121ms, spatial resolution=0.45x0.45x4mm^3) images were acquired as part of the larger multimodal neuroimaging protocol. As previously published (Jefferson et al., 2016), FLAIR images were post-processed using the Lesion Segmentation Tool toolbox for Statistical Parametric Mapping (SPM8) (Schmidt et al., 2012) excluding the cerebellum and brainstem. FLAIR images were bias-corrected for field inhomogeneities and registered to the T1-weighted images. FLAIR intensity distribution of white matter, gray matter, and CSF were assigned, enabling detection of outliers. Neighboring voxels were classified iteratively and analyzed and assigned to lesion, white matter, or gray matter until no more voxels were assigned to a lesion. Scans were individually reviewed and manually corrected for any mislabeling. Manual corrections were then confirmed by a board-certified neuroradiologist blinded to clinical information (LTD) using the Medical Image Processing, Analysis, and Visualization application (http://mipav.cit.nih.gov). Intracranial volume was calculated based on a summation of participant-specific grey matter, white matter, and CSF using T1-weighted images with SPM8.

2.4 Analytical Plan

Systolic blood pressure was the mean of two measurements obtained prior to the echocardiogram. Diabetes mellitus was defined as current fasting blood glucose ≥126 mg/dL, hemoglobin A1C≥6.5%, or current oral hypoglycemic or insulin medication usage. Medication review determined anti-hypertensive medication use. Left ventricular

hypertrophy was defined on echocardiogram as left ventricle mass index >115 g/m^2 in men or >95 g/m^2 in women. Self-report atrial fibrillation was corroborated by echocardiogram, cardiac magnetic resonance imaging, documentation of prior procedure/ablation for atrial fibrillation, or medication usage for atrial fibrillation. Current cigarette smoking (yes/no within the year prior to baseline examination) was ascertained by self-report. Self-report prevalent cardiovascular disease with supporting evidence from medical records included coronary heart disease, angina, or myocardial infarction (note, heart failure was an exclusion for the parent study). Framingham Stroke Risk Profile (FSRP) score was calculated by applying points by sex for age, systolic blood pressure accounting for anti-hypertensive medication usage, diabetes mellitus, current cigarette smoking, left ventricular hypertrophy, prevalent cardiovascular disease, and atrial fibrillation (D'Agostino et al., 1994). We excluded points assigned for age since age was included as a separate covariate in our statistical models. *APOE* genotyping was performed on whole blood samples. Apolipoprotein E ε4 (*APOE4*) status was defined as positive (ε2/ε4, ε3/ε4, ε4/ε4) or negative (ε2/ε2, ε2/ε3, ε3/ε3). *APOE2* status was defined as positive ε2/ε2, ε2/ε3, ε4/ε4) or negative (ε3/ε3, ε3/ε4, ε4/ε4). Intracranial volume was calculated using methods described above. WMHs (cm^3) were log-transformed prior to analyses.

Unadjusted Spearman rank correlations among CSF biomarkers were assessed. For hypothesis testing, linear regression models with ordinary least square estimates related each CSF biomarker ($A\beta_{42}$, p-tau, t-tau, and NFL) to log WMHs. Models were adjusted for age, sex, race/ethnicity, education, intracranial volume, cognitive diagnosis, and *APOE4*. Given extensive literature suggesting vascular risk factors increase the risk

of WMHs (Fazekas et al., 1988; Lazarus et al., 2005) and clinical AD (Borenstein et al., 2005; Kivipelto et al., 2005), models were also adjusted for adverse vascular risk using a modified version (excluding points assigned for age) of the FSRP (D'Agostino et al., 1994) to avoid arbitrarily detecting a connection between $A\beta_{42}$ and WMHs that might be due to shared vascular risk factors. A post-hoc linear regression model related those CSF biomarkers with significant associations to WMHs as competing predictors using identical covariates as the primary models. This competitive model approach assessed the extent to which the variance accounted for by an individual biomarker was unique or overlapping with another biomarker. Competitive models were repeated including all remaining CSF biomarkers as additional covariates in separate models. Post-hoc linear regression models related an $A\beta_{42}$ x NFL interaction term to WMHs. $A\beta_{42}$ interaction terms were also separately run for age, *APOE4* allele status (dichotomized by presence of at least one ε4 allele), *APOE2* allele status (dichotomized by presence of at least one ε2 allele), and cognitive diagnosis (NC, MCI) on WMHs. Each model with $A\beta_{42}$ as the predictor was repeated including NFL, NFL + p-tau, and NFL + t-tau as covariates in separate models. All models were then repeated stratified by cognitive diagnosis (NC, MCI). Significance was set *a priori* at $p<0.05$. Analyses were conducted using R 3.1.2 (www.r-project.org).

3. Results

3.1 Participant & CSF Biomarker Characteristics

Participants included 148 adults age 60 to 90 years (72±6 years), 67% were men, and 93% self-identified as non-Hispanic White. $A\beta_{42}$ levels correlated with p-tau

(r=-0.21, p<0.01) and t-tau levels (r=-0.25, p<0.001). P-tau and t-tau levels correlated (r=0.98, p<0.001). NFL levels correlated with p-tau (r=0.51, p<0.001) and t-tau levels (r=0.57, p<0.001). NFL was not associated with $A\beta_{42}$ (r=-0.06, p=0.49). Participant and biomarker characteristics are presented in **Table 1**.

3.2 CSF Biomarkers as Individual & Interactive Predictors of WMHs

In linear regression models, $A\beta_{42}$ concentrations (p=0.003) and NFL concentrations (p=0.02) related to WMHs. $A\beta_{42}$ did not interact with NFL on WMHs (p=0.34). Neither p-tau nor t-tau concentrations were significantly related to WMHs (p-values>0.21). See **Table 2** for effect sizes and **Figure 3** for illustrations.

Aß$_{42}$ interacted with cognitive diagnosis on WMHs (p=0.03), a finding that persisted when NFL was added as a covariate (p=0.009). Stratified analyses revealed $A\beta_{42}$ related to WMHs only in the NC participants (NC p=0.001; MCI p=0.31), which also persisted when NFL was added as a covariate (p=0.0004). $A\beta_{42}$ did not interact with age (p=0.88), APOE4 allele status (p=0.50), or APOE2 allele status (p=0.13) on WMHs. These interaction models remained null when NFL was added as a covariate (all p-values>0.09). NFL did not interact with cognitive diagnosis (p=0.68), age (p=0.83), APOE4 allele status (p=0.63), or APOE2 allele status (p=0.52) on WMHs. Results were similar when including p-tau or t-tau as an additional covariate. See **Table 3** for details.

3.3 CSF Biomarkers as Competing Predictors of WMHs

In a combined model including the two CSF biomarkers with associations with WMHs (i.e., $A\beta_{42}$ and NFL), both $A\beta_{42}$ (p<0.001) and NFL concentrations (p=0.006) related to WMHs. Covariates accounted for 24.8% of the variance, including 0.5% from the modified FSRP. When $A\beta_{42}$ was added to the model containing just the covariates, it

accounted for an additional 3.9% unique variance in WMHs (p=0.007). Adding NFL to the model containing just the covariates contributed an additional 3.2% unique variance (p=0.01). Adding $A\beta_{42}$ to the model already containing covariates and NFL contributed an additional 4.3% unique variance, resulting in 7.5% combined variance accounted for by $A\beta_{42}$ and NFL beyond covariates. See **Table 2** for details.

4. Discussion

Among community dwelling older adults free of clinical dementia and stroke, we found lower CSF $A\beta_{42}$ and higher CSF NFL related to increased WMHs. When CSF $A\beta_{42}$ and NFL were included as dual predictors in a single model, each biomarker statistically accounted for unique variance. However, there was no significant Aβ42 x NFL interaction on WMHs. The novelty of this study lies in the use of a single, integrated analytical model to concurrently relate multiple CSF biomarkers to WMHs assessing the extent to which the variance accounted for by each biomarker is overlapping with another biomarker. Our inclusion of an older adult cohort, for which two-third of participants were cognitively normal, also addresses a crucial gap in the literature regarding the need for more comprehensive biomarker models to predict AD and related neurodegeneration in preclinical populations. Prior work has focused primarily on the utility of NFL as an individual biomarker of disease progression in clinical cohorts. By examining NFL in a competitive analytical model among a non-demented and predominantly cognitively normal sample, we critically extend the literature regarding the additive value of NFL as part of an integrated biomarker model in the neurobiology of aging adults. Although cross-sectional, results provide initial

evidence suggesting WMHs may reflect distinct neuropathological pathways, including both amyloid aggregation and axonal injury.

Our observation that CSF $A\beta_{42}$ relates to WMHs (Marnane et al., 2016) after adjusting for common confounds, including systemic vascular risk and genetic susceptibility to AD, strengthens the hypothesis that an independent Aβ pathway contributes to WMHs. The mechanistic link between amyloid and WMHs remains elusive but may reflect impaired amyloid clearance through perivascular interstitial fluid drainage pathways (Iliff et al., 2012; Kress et al., 2014). Emerging evidence suggests drainage systems within the basement membrane of cerebral vessels constitute important clearance pathways for amyloid. Aβ particles are prone to aggregate and cohere to membrane surfaces, causing them to become trapped alongside immune complexes, barricading flow of interstitial fluid (Mawuenyega et al., 2010; Potter et al., 2013; Zekonyte et al., 2016). This process may result in fluid accumulation and white matter disruption appearing as WMHs on FLAIR, with co-occurring evidence of cerebral amyloid deposition (Weller et al., 2015). **Figure 1** illustrates functional and pathological versions of this neural system by which waste products, including insoluble Aβ particles, are cleared into interstitial fluid for ultimate removal through the CSF.

Another important observation from this study is that CSF NFL is associated with WMHs even after adjusting for $A\beta_{42}$ levels, suggesting an axonal injury pathway for WMHs unrelated to β-amyloid. Both CSF NFL (Jonsson et al., 2010) and WMHs (Molad et al., 2017; Wardlaw et al., 2013) have been linked to small vessel disease, suggesting small vessel disease may underlie this association. Our older adult sample was free of clinical stroke with limited prevalent cardiovascular disease, and analytical models

statistically accounted for systemic vascular risk factors. Therefore, it is unlikely that small vessel disease fully accounted for the observed association reported here. The pathology underlying WMHs is not completely known and requires further research for a more comprehensive understanding. A second possibility is that the co-occurrence of axonal damage (represented by increased CSF NFL) and increased WMHs represents a heterogeneous set of underlying disease states that result in substantial damage to white matter fiber tracts. Regardless of mechanism, CSF NFL elevations correspond temporally with acute axonal injury (Bacioglu et al., 2016), so WMHs among older adults may signify an active process of white matter tissue breakdown. Given the lack of consistent findings regarding histopathological correlates of WMHs (Black et al., 2009; Shoamanesh et al., 2011), future studies should longitudinally track temporal concordance between changes in CSF NFL and WMHs. Such research could improve understanding of whether WMHs persist following a return to baseline in CSF NFL concentrations with remission of active white matter injury processes, and whether WMHs may represent some threshold of clinical importance regarding CSF NFL elevations.

Consistent with prior literature, neither t-tau (Kester et al., 2014) nor p-tau (Guzman et al., 2013) related to WMHs in our sample. Prior literature relating tau expression to early axonal injury suggests these associations tend to initiate in the perforant pathway and in unmyelinated axons within cortical tissue. Tau associations with large-caliber white matter tract damage then occur later in the disease after clinical symptoms manifest. In contrast, NFL reflects structural axonal damage regardless of disease stage. Thus, in our community-based sample free of clinical dementia, it is not

surprising that NFL related more closely to WMHs than tau, since tau expression at this preclinical stage is likely more reflective of upstream changes to neuronal health that may not yet be sufficient to impact structural axonal damage visible on white matter imaging. Underlying mechanisms relating tau protein to white matter damage remain unclear, and further study is warranted to elucidate pathogenic substrates of various tau biomarkers.

Our cohort was comprised of older adults free of clinical dementia and stroke, two-thirds of whom were cognitively normal. Utilization of a dementia free cohort addresses a crucial gap in the literature regarding the need for more comprehensive biomarker models to predict AD in preclinical populations. Prior work investigating NFL has focused primarily on its utility as an individual biomarker of disease progression in clinical populations. By examining NFL in a competitive analytical model among a non-demented and predominantly cognitively normal sample, we critically extend the literature regarding the additive value of NFL as part of an integrated biomarker model among a preclinical aging population.

The current study has several strengths. First, MRI data were collected on a single research scanner using T2 FLAIR gold-standard methods for detecting WMHs. Both neuroimaging and CSF measurements were processed in core laboratories where raters were blinded to clinical information. Potential confounders were ascertained in a comprehensive manner, including key covariates that increase cerebral small vessel disease and clinical AD risk.

Despite these strengths, several study limitations are noteworthy, including the cross-sectional nature of the methods, which limits drawing conclusions about potential

underlying mechanisms. Other limitations include a relatively homogenous participant sample in terms of race/ethnicity, and lack of direct method to quantify interstitial fluid drainage impairments. Also, covariates in our model accounted for 28.5% of the variance in WMHs, and $A\beta_{42}$ and NFL accounted for an additional 7.5% beyond that. Thus, the majority of the variance in WMHs in our sample is due to unknown factors. This large proportion of unexplained variance limits our ability to draw mechanistic conclusions and speaks to the heterogeneity and ambiguity of WMH substrates in older adults, representing an important area for further study. Given the cohort sample characteristics (e.g., carefully screened community-based cohort without clinical stroke or dementia, most of whom were cognitively normal with a relatively low burden of prevalent cardiovascular disease) we would not expect to see a high degree of explained variance in WMHs. We speculate findings might become more pronounced among more clinically diverse samples of individuals with poorer health characteristics and more advanced neurodegenerative disease, representing an important area of future investigation. Future research should also explore other biomarkers reflecting different pathways of interest (e.g., inflammation, extracellular matrix), which may relate to WMHs and provide further insight into the pathological processes underlying WMHs.

In summary, our findings support the co-occurrence of at least two substrates of WMHs among older adults, including distinct amyloid and non-amyloid pathways. This observation in a dementia-free sample enriched for mild cognitive impairment suggests heterogeneous etiologies underlie development of WMHs prior to clinical manifestation of AD and related dementia. Our work highlights the importance of investigating neurodegenerative markers in integrative, competitive models rather than studying

inherently connected variables in an isolated ‘silo’ approach (Jefferson, 2014). It is essential that future research investigate WMHs and emerging dynamic biomarkers to better understand the relative contributions of vascular changes or problems (including both amyloid and non-amyloid pathways), *ex vacuo* fluid accumulation from neurodegeneration, axonal damage and corresponding demyelination, and other factors yet to be identified in the development of WMHs. Specifically, future research should investigate possible neuropathological mechanisms underlying the link between CSF NFL and WMHs, and leverage human data to corroborate animal models suggesting impaired interstitial fluid clearance of $A\beta_{42}$ may account for the amyloid-dependent WMHs pathway we observed. Finally, future studies should employ longitudinal designs to elucidate how CSF biomarkers relate to changes in WMHs over time and in conjunction with clinical outcomes, including cognitive decline.

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7. References

Aisen, P.S., Petersen, R.C., Donohue, M.C., Gamst, A., Raman, R., Thomas, R.G., Walter, S., Trojanowski, J.Q., Shaw, L.M., Beckett, L.A., Jack, C.R., Jr., Jagust, W., Toga, A.W., Saykin, A.J., Morris, J.C., Green, R.C., Weiner, M.W., Alzheimer's Disease Neuroimaging, I., 2010. Clinical Core of the Alzheimer's Disease Neuroimaging Initiative: progress and plans. Alzheimers Dement 6(3), 239-246.

Albert, M.S., DeKosky, S.T., Dickson, D., Dubois, B., Feldman, H.H., Fox, N.C., Gamst, A., Holtzman, D.M., Jagust, W.J., Petersen, R.C., Snyder, P.J., Carrillo, M.C., Thies, B., Phelps, C.H., 2011. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's and Dementia 7(3), 270-279.

Bacioglu, M., Maia, L.F., Preische, O., Schelle, J., Apel, A., Kaeser, S.A., Schweighauser, M., Eninger, T., Lambert, M., Pilotto, A., Shimshek, D.R., Neumann, U., Kahle, P.J., Staufenbiel, M., Neumann, M., Maetzler, W., Kuhle, J., Jucker, M., 2016. Neurofilament Light Chain in Blood and CSF as Marker of Disease Progression in Mouse Models and in Neurodegenerative Diseases. Neuron 91(2), 494-496.

Benedictus, M.R., van Harten, A.C., Leeuwis, A.E., Koene, T., Scheltens, P., Barkhof, F., Prins, N.D., van der Flier, W.M., 2015. White Matter Hyperintensities Relate to Clinical Progression in Subjective Cognitive Decline. Stroke 46(9), 2661-2664.

Bjerke, M., Jonsson, M., Nordlund, A., Eckerstrom, C., Blennow, K., Zetterberg, H., Pantoni, L., Inzitari, D., Schmidt, R., Wallin, A., 2014. Cerebrovascular Biomarker Profile

Is Related to White Matter Disease and Ventricular Dilation in a LADIS Substudy. Dement. Geriatr. Cogn. Dis. Extra 4(3), 385-394.

Black, S., Gao, F., Bilbao, J., 2009. Understanding white matter disease: imaging-pathological correlations in vascular cognitive impairment. Stroke 40(3 Suppl), S48-52.

Blennow, K., Hampel, H., Weiner, M., Zetterberg, H., 2010. Cerebrospinal fluid and plasma biomarkers in Alzheimer's disease. Nat Rev Neurol 6, 131-144.

Borenstein, A.R., Wu, Y., Mortimer, J.A., Schellenberg, G.D., McCormick, W.C., Bowen, J.D., McCurry, S., Larson, E.B., 2005. Developmental and vascular risk factors for Alzheimer's disease. Neurobiol. Aging 26(3), 325-334.

Buerger, K., Ewers, M., Pirttila, T., Zinkowski, R., Alafuzoff, I., Teipel, S.J., DeBernardis, J., Kerkman, D., McCulloch, C., Soininen, H., Hampel, H., 2006. CSF phosphorylated tau protein correlates with neocortical neurofibrillary pathology in Alzheimer's disease. Brain 129(Pt 11), 3035-3041.

D'Agostino, R.B., Wolf, P.A., Belanger, A.J., Kannel, W.B., 1994. Stroke risk profile: adjustment for antihypertensive medication. The Framingham Study. Stroke 25(1), 40-43.

Debette, S., Beiser, A., DeCarli, C., Au, R., Himali, J.J., Kelly-Hayes, M., Romero, J.R., Kase, C.S., Wolf, P.A., Seshadri, S., 2010. Association of MRI markers of vascular brain injury with incident stroke, mild cognitive impairment, dementia, and mortality: the Framingham Offspring Study. Stroke 41(4), 600-606.

Erten-Lyons, D., Woltjer, R., Kaye, J., Mattek, N., Dodge, H.H., Green, S., Tran, H., Howieson, D.B., Wild, K., Silbert, L.C., 2013. Neuropathologic basis of white matter hyperintensity accumulation with advanced age. Neurology 81(11), 977-983.

Fazekas, F., Niederkorn, K., Schmidt, R., Offenbacher, H., Horner, S., Bertha, G., Lechner, H., 1988. White matter signal abnormalities in normal individuals: correlation with carotid ultrasonography, cerebral blood flow measurements, and cerebrovascular risk factors. Stroke 19(10), 1285-1288.

Garde, E., Mortensen, E.L., Krabbe, K., Rostrup, E., Larsson, H.B., 2000. Relation between age-related decline in intelligence and cerebral white-matter hyperintensities in healthy octogenarians: a longitudinal study. Lancet 356(9230), 628-634.

Gouw, A.A., Seewann, A., van der Flier, W.M., Barkhof, F., Rozemuller, A.M., Scheltens, P., Geurts, J.J., 2011. Heterogeneity of small vessel disease: a systematic review of MRI and histopathology correlations. Journal of Neurology, Neurosurgery, and Psychiatry 82(2), 126-135.

Grimmer, T., Faust, M., Auer, F., Alexopoulos, P., Forstl, H., Henriksen, G., Perneczky, R., Sorg, C., Yousefi, B.H., Drzezga, A., Kurz, A., 2012. White matter hyperintensities predict amyloid increase in Alzheimer's disease. Neurobiol. Aging 33(12), 2766-2773.

Guzman, V.A., Carmichael, O.T., Schwarz, C., Tosto, G., Zimmerman, M.E., Brickman, A.M., 2013. White matter hyperintensities and amyloid are independently associated with entorhinal cortex volume among individuals with mild cognitive impairment. Alzheimers Dement 9(5 Suppl), S124-131.

Hertze, J., Palmqvist, S., Minthon, L., Hansson, O., 2013. Tau pathology and parietal white matter lesions have independent but synergistic effects on early development of Alzheimer's disease. Dement. Geriatr. Cogn. Dis. Extra 3(1), 113-122.

Iliff, J.J., Wang, M., Liao, Y., Plogg, B.A., Peng, W., Gundersen, G.A., Benveniste, H., Vates, G.E., Deane, R., Goldman, S.A., Nagelhus, E.A., Nedergaard, M., 2012. A

paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid beta. Sci. Transl. Med. 4(147), 147ra111.

Jagust, W.J., Zheng, L., Harvey, D.J., Mack, W.J., Vinters, H.V., Weiner, M.W., Ellis, W.G., Zarow, C., Mungas, D., Reed, B.R., Kramer, J.H., Schuff, N., DeCarli, C., Chui, H.C., 2008. Neuropathological basis of magnetic resonance images in aging and dementia. Ann. Neurol. 63(1), 72-80.

Jefferson, A.L., 2014. Vascular risk factors and midlife cognition: rethinking the exposure window. Circulation 129(15), 1548-1550.

Jefferson, A.L., Gifford, K.A., Acosta, L.M., Bell, S.P., Donahue, M.J., Taylor Davis, L., Gottlieb, J., Gupta, D.K., Hohman, T.J., Lane, E.M., Libon, D.J., Mendes, L.A., Niswender, K., Pechman, K.R., Rane, S., Ruberg, F.L., Ru Su, Y., Zetterberg, H., Liu, D., 2016. The Vanderbilt Memory & Aging Project: Study Design and Baseline Cohort Overview. Journal of Alzheimer's Disease 52(2), 539-559.

Jonsson, M., Zetterberg, H., van Straaten, E., Lind, K., Syversen, S., Edman, A., Blennow, K., Rosengren, L., Pantoni, L., Inzitari, D., Wallin, A., 2010. Cerebrospinal fluid biomarkers of white matter lesions - cross-sectional results from the LADIS study. Eur. J. Neurol. 17(3), 377-382.

Kandel, B.M., Avants, B.B., Gee, J.C., McMillan, C.T., Erus, G., Doshi, J., Davatzikos, C., Wolk, D.A., 2016. White matter hyperintensities are more highly associated with preclinical Alzheimer's disease than imaging and cognitive markers of neurodegeneration. Alzheimer's & dementia (Amsterdam, Netherlands) 4, 18-27.

Kester, M.I., Goos, J.D., Teunissen, C.E., Benedictus, M.R., Bouwman, F.H., Wattjes, M.P., Barkhof, F., Scheltens, P., van der Flier, W.M., 2014. Associations between cerebral small-vessel disease and Alzheimer disease pathology as measured by cerebrospinal fluid biomarkers. JAMA Neurol 71(7), 855-862.

Kivipelto, M., Ngandu, T., Fratiglioni, L., Viitanen, M., Kareholt, I., Winblad, B., Helkala, E.L., Tuomilehto, J., Soininen, H., Nissinen, A., 2005. Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. Arch. Neurol. 62(10), 1556-1560.

Kress, B.T., Iliff, J.J., Xia, M., Wang, M., Wei, H.S., Zeppenfeld, D., Xie, L., Kang, H., Xu, Q., Liew, J.A., Plog, B.A., Ding, F., Deane, R., Nedergaard, M., 2014. Impairment of paravascular clearance pathways in the aging brain. Ann. Neurol. 76(6), 845-861.

Lazarus, R., Prettyman, R., Cherryman, G., 2005. White matter lesions on magnetic resonance imaging and their relationship with vascular risk factors in memory clinic attenders. Int. J. Geriatr. Psychiatry 20(3), 274-279.

Lee, S., Viqar, F., Zimmerman, M.E., Narkhede, A., Tosto, G., Benzinger, T.L., Marcus, D.S., Fagan, A.M., Goate, A., Fox, N.C., Cairns, N.J., Holtzman, D.M., Buckles, V., Ghetti, B., McDade, E., Martins, R.N., Saykin, A.J., Masters, C.L., Ringman, J.M., Ryan, N.S., Forster, S., Laske, C., Schofield, P.R., Sperling, R.A., Salloway, S., Correia, S., Jack, C., Jr., Weiner, M., Bateman, R.J., Morris, J.C., Mayeux, R., Brickman, A.M., 2016. White matter hyperintensities are a core feature of Alzheimer's disease: Evidence from the dominantly inherited Alzheimer network. Ann. Neurol. 79(6), 929-939.

Marnane, M., Al-Jawadi, O.O., Mortazavi, S., Pogorzelec, K.J., Wang, B.W., Feldman, H.H., Hsiung, G.Y., 2016. Periventricular hyperintensities are associated with elevated cerebral amyloid. Neurology 86(6), 535-543.

Mawuenyega, K.G., Sigurdson, W., Ovod, V., Munsell, L., Kasten, T., Morris, J.C., Yarasheski, K.E., Bateman, R.J., 2010. Decreased clearance of CNS beta-amyloid in Alzheimer's disease. Science 330(6012), 1774.

McAleese, K.E., Firbank, M., Dey, M., Colloby, S.J., Walker, L., Johnson, M., Beverley, J.R., Taylor, J.P., Thomas, A.J., O'Brien, J.T., Attems, J., 2015. Cortical tau load is associated with white matter hyperintensities. Acta neuropathologica communications 3, 60.

Molad, J., Kliper, E., Korczyn, A.D., Ben Assayag, E., Ben Bashat, D., Shenhar-Tsarfaty, S., Aizenstein, O., Shopin, L., Bornstein, N.M., Auriel, E., 2017. Only White Matter Hyperintensities Predicts Post-Stroke Cognitive Performances Among Cerebral Small Vessel Disease Markers: Results from the TABASCO Study. J Alzheimers Dis 56(4), 1293-1299.

Olsson, B., Lautner, R., Andreasson, U., Ohrfelt, A., Portelius, E., Bjerke, M., Holtta, M., Rosen, C., Olsson, C., Strobel, G., Wu, E., Dakin, K., Petzold, M., Blennow, K., Zetterberg, H., 2016. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Lancet Neurol 15(7), 673-684.

Palmqvist, S., Zetterberg, H., Blennow, K., Vestberg, S., Andreasson, U., Brooks, D.J., Owenius, R., Hagerstrom, D., Wollmer, P., Minthon, L., Hansson, O., 2014. Accuracy of brain amyloid detection in clinical practice using cerebrospinal fluid beta-amyloid 42: a

cross-validation study against amyloid positron emission tomography. JAMA neurology 71(10), 1282-1289.

Potter, R., Patterson, B.W., Elbert, D.L., Ovod, V., Kasten, T., Sigurdson, W., Mawuenyega, K., Blazey, T., Goate, A., Chott, R., Yarasheski, K.E., Holtzman, D.M., Morris, J.C., Benzinger, T.L., Bateman, R.J., 2013. Increased in vivo amyloid-beta42 production, exchange, and loss in presenilin mutation carriers. Sci. Transl. Med. 5(189), 189ra177.

Provenzano, F.A., Muraskin, J., Tosto, G., Narkhede, A., Wasserman, B.T., Griffith, E.Y., Guzman, V.A., Meier, I.B., Zimmerman, M.E., Brickman, A.M., 2013. White matter hyperintensities and cerebral amyloidosis: necessary and sufficient for clinical expression of Alzheimer disease? JAMA Neurol 70(4), 455-461.

Pruessmann, K.P., Weiger, M., Scheidegger, M.B., Boesiger, P., 1999. SENSE: sensitivity encoding for fast MRI. Magn. Reson. Med. 42(5), 952-962.

Schmidt, P., Gaser, C., Arsic, M., Buck, D., Forschler, A., Berthele, A., Hoshi, M., Ilg, R., Schmid, V.J., Zimmer, C., Hemmer, B., Muhlau, M., 2012. An automated tool for detection of FLAIR-hyperintense white-matter lesions in Multiple Sclerosis. Neuroimage 59(4), 3774-3783.

Seppala, T.T., Nerg, O., Koivisto, A.M., Rummukainen, J., Puli, L., Zetterberg, H., Pyykko, O.T., Helisalmi, S., Alafuzoff, I., Hiltunen, M., Jaaskelainen, J.E., Rinne, J., Soininen, H., Leinonen, V., Herukka, S.K., 2012. CSF biomarkers for Alzheimer disease correlate with cortical brain biopsy findings. Neurology 78(20), 1568-1575.

Shoamanesh, A., Kwok, C.S., Benavente, O., 2011. Cerebral microbleeds: histopathological correlation of neuroimaging. Cerebrovasc. Dis. 32(6), 528-534.

Skillback, T., Farahmand, B., Bartlett, J.W., Rosen, C., Mattsson, N., Nagga, K., Kilander, L., Religa, D., Wimo, A., Winblad, B., Rosengren, L., Schott, J.M., Blennow, K., Eriksdotter, M., Zetterberg, H., 2014. CSF neurofilament light differs in neurodegenerative diseases and predicts severity and survival. Neurology 83(21), 1945-1953.

Tosto, G., Zimmerman, M.E., Hamilton, J.L., Carmichael, O.T., Brickman, A.M., 2015. The effect of white matter hyperintensities on neurodegeneration in mild cognitive impairment. Alzheimers Dement 11(12), 1510-1519.

van Westen, D., Lindqvist, D., Blennow, K., Minthon, L., Nagga, K., Stomrud, E., Zetterberg, H., Hansson, O., 2016. Cerebral white matter lesions - associations with Abeta isoforms and amyloid PET. Sci. Rep. 6, 20709.

Wardlaw, J.M., Smith, E.E., Biessels, G.J., Cordonnier, C., Fazekas, F., Frayne, R., Lindley, R.I., O'Brien, J.T., Barkhof, F., Benavente, O.R., Black, S.E., Brayne, C., Breteler, M., Chabriat, H., Decarli, C., de Leeuw, F.E., Doubal, F., Duering, M., Fox, N.C., Greenberg, S., Hachinski, V., Kilimann, I., Mok, V., Oostenbrugge, R., Pantoni, L., Speck, O., Stephan, B.C., Teipel, S., Viswanathan, A., Werring, D., Chen, C., Smith, C., van Buchem, M., Norrving, B., Gorelick, P.B., Dichgans, M., 2013. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurology 12(8), 822-838.

Weller, R.O., Hawkes, C.A., Kalaria, R.N., Werring, D.J., Carare, R.O., 2015. White matter changes in dementia: role of impaired drainage of interstitial fluid. Brain Pathol. 25(1), 63-78.

Ylikoski, A., Erkinjuntti, T., Raininko, R., Sarna, S., Sulkava, R., Tilvis, R., 1995. White matter hyperintensities on MRI in the neurologically nondiseased elderly. Analysis of cohorts of consecutive subjects aged 55 to 85 years living at home. Stroke 26(7), 1171-1177.

Zekonyte, J., Sakai, K., Nicoll, J.A., Weller, R.O., Carare, R.O., 2016. Quantification of molecular interactions between ApoE, amyloid-beta (Abeta) and laminin: Relevance to accumulation of Abeta in Alzheimer's disease. Biochim. Biophys. Acta 1862(5), 1047-1053.

Table 1. Participant Characteristics

	n=148
Demographics & Health Characteristics	
Age, years	72±6
Sex, % male	67
Race, % White Non-Hispanic	93
Education, years	16±3
Cognitive diagnosis, % mild cognitive impairment	38
Montreal Cognitive Assessment, total	26±3
APOE4, % carriers	33
APOE4, % allelles	
ε2/ε2	1
ε2/ε3	8
ε2/ε4	2
ε3/ε3	58
ε3/ε4	22
ε4/ε4	9
Framingham Stroke Risk Profile, total*	12±4
Systolic blood pressure, mmHg	142±16
Anti-hypertensive medication usage, %	46
Diabetes, %	17
Cigarette smoking current, %	1
Prevalent cardiovascular disease, %	3
Atrial fibrillation, %	3
Left ventricular hypertrophy, %	3
CSF Biomarker Status & Concentrations, pg/mL	
$A\beta_{42}$	718±244
Amyloid positive (≤530), %	29
T-tau	427±228
T-tau positive (≥400), %	43
P-tau[ł]	61±26
Neurofilament Light[ł]	1068±581
Neuroimaging Characteristics	
Intracranial volume, cm^3	1409±128
Raw WMHs, cm^3	15±19
Log-transformed WMHs	2.12±1.13

Note. Values denoted as mean±standard deviation or frequency; *a modified FSRP score was included in statistical models excluding points assigned to age (6.1±2.6); [ł]no cut-point available; *APOE4*=apolipoprotein E ε4 allele; WMHs=white matter

hyperintensities

Table 2. Main Effect & Interaction Model Results

	R^2	R^2 adjusted	*Δ R^2 adjusted	f^2	Partial R^2	**P-value
†Covariates +						
Aß$_{42}$	0.29	0.21	0.02	0.03	0.03	0.007
T-tau	0.25	0.19	-0.006	<0.0001	<0.0001	0.34
P-tau	0.25	0.19	-0.004	0.002	0.002	0.61
NFL	0.28	0.22	0.02	0.04	0.03	0.01
Aß$_{42}$ x NFL	0.31	0.24	0.03	0.06	0.05	0.36
Aß$_{42}$ x age	0.28	0.20	-0.006	0.0001	0.0001	0.71
Aß$_{42}$ x *APOE* ε4	0.28	0.21	-0.003	0.003	0.003	0.65
Aß$_{42}$ x *APOE* ε2	0.30	0.23	0.02	0.04	0.03	0.10
Aß$_{42}$ x diagnosis	0.30	0.23	0.02	0.04	0.04	0.03
Covariates + NFL +						
Aß$_{42}$	0.31	0.24	0.03	0.04	0.04	0.02
T-tau	0.28	0.21	-0.004	0.003	0.003	0.55
P-tau	0.28	0.22	-0.0002	0.007	0.007	0.33
Aß$_{42}$ x age	0.31	0.24	-0.004	0.003	0.003	0.55
Aß$_{42}$ x *APOE* ε4	0.31	0.24	0.002	0.005	0.005	0.42
Aß$_{42}$ x *APOE* ε2	0.33	0.26	0.02	0.04	0.04	0.10
Aß$_{42}$ x diagnosis	0.34	0.27	0.03	0.05	0.05	0.01
NFL x age	0.28	0.22	-0.006	<0.0001	<0.0001	1.00
NFL x *APOE* ε4	0.28	0.22	-0.006	0.0002	0.0002	0.87
NFL x *APOE* ε2	0.29	0.23	-0.001	0.006	0.006	0.43
NFL x diagnosis	0.29	0.23	0.002	0.01	0.01	0.62
Covariates + NFL + P-tau +						
Aß$_{42}$	0.31	0.24	0.03	0.04	0.04	0.02
T-tau	0.29	0.21	0.0005	0.007	0.007	0.34
Aß$_{42}$ x age	0.32	0.24	-0.004	0.002	0.002	0.63
Aß$_{42}$ x *APOE* ε4	0.32	0.24	-0.001	0.006	0.006	0.37
Aß$_{42}$ x *APOE* ε2	0.34	0.26	0.02	0.03	0.03	0.11
Aß$_{42}$ x diagnosis	0.34	0.27	0.02	0.05	0.04	0.01
Covariates + NFL + T-tau +						
Aß$_{42}$	0.31	0.24	0.03	0.06	0.04	0.02
Aß$_{42}$ x age	0.31	0.24	-0.004	0.002	0.002	0.61
Aß$_{42}$ x *APOE* ε4	0.32	0.24	0.001	0.006	0.006	0.37
Aß$_{42}$ x *APOE* ε2	0.34	0.26	0.02	0.03	0.03	0.10
Aß$_{42}$ x diagnosis	0.34	0.26	0.03	0.05	0.04	0.01

Note. *Represents change in R^2 from the covariates only model; **For predictor(s) after covariates entered into the model; †includes age, education, sex, race/ethnicity, intracranial

volume, modified Framingham Stroke Risk Profile with points excluded for age, cognitive diagnosis, and separately for *APOE4* and *APOE2* carrier status; All analyses were re-run excluding predictor outliers > 4 standard deviations from mean (n=3), and there were no significant changes in results; NFL=neurofilament light

Table 3. Model Results Stratified by Cognitive Diagnosis

	R^2	R^2 adjusted	*Δ R^2 adjusted	f^2	Partial R^2	**P-value
Cognitively Normal						
†Covariates +						
Aß$_{42}$	0.37	0.27	0.05	0.08	0.08	0.02
T-tau	0.34	0.24	0.02	0.04	0.04	0.09
P-tau	0.34	0.25	0.03	0.05	0.05	0.07
NFL	0.31	0.21	-0.009	0.004	0.004	0.62
Covariates + NFL +						
Aß$_{42}$	0.38	0.27	0.06	0.10	0.09	0.01
T-tau	0.35	0.24	0.03	0.05	0.05	0.07
P-tau	0.35	0.24	0.03	0.05	0.05	0.07
Covariates + NFL + P-tau +						
Aß$_{42}$	0.39	0.28	0.04	0.07	0.07	0.04
T-tau	0.35	0.23	-0.01	0.002	0.002	0.71
Covariates + NFL + T-tau +						
Aß$_{42}$	0.40	0.28	0.04	0.08	0.07	0.03
Mild Cognitive Impairment						
Covariates +						
Aß$_{42}$	0.27	0.11	-0.004	0.02	0.02	0.38
T-tau	0.31	0.15	0.04	0.07	0.07	0.08
P-tau	0.29	0.13	0.02	0.04	0.04	0.17
NFL	0.35	0.20	0.09	0.13	0.12	0.02
Covariates + NFL +						
Aß$_{42}$	0.35	0.19	-0.009	0.01	0.01	0.48
T-tau	0.36	0.21	0.006	0.03	0.03	0.26
P-tau	0.36	0.19	-0.006	0.02	0.01	0.42
Covariates + NFL + P-tau +						
Aß$_{42}$	0.36	0.18	-0.009	0.01	0.01	0.48
T-tau	0.37	0.20	0.007	0.03	0.03	0.25
Covariates + NFL + T-tau +						
Aß$_{42}$	0.37	0.20	-0.007	0.01	0.01	0.44

Note. *Represents change in R^2 from the covariates only model; **For predictor(s) after covariates entered into the model; †includes age, education, sex, race/ethnicity, intracranial volume, modified Framingham Stroke Risk Profile with points excluded for age, cognitive diagnosis, and *APOE4* carrier status; All analyses were re-run excluding predictor outliers > 4 standard deviations from mean, and there were no significant changes in results; NFL=neurofilament light; p-tau=hyperphosphorylated tau; t-tau=total tau.

Figure 1. Pathophysiology Associated with CSF Biomarkers

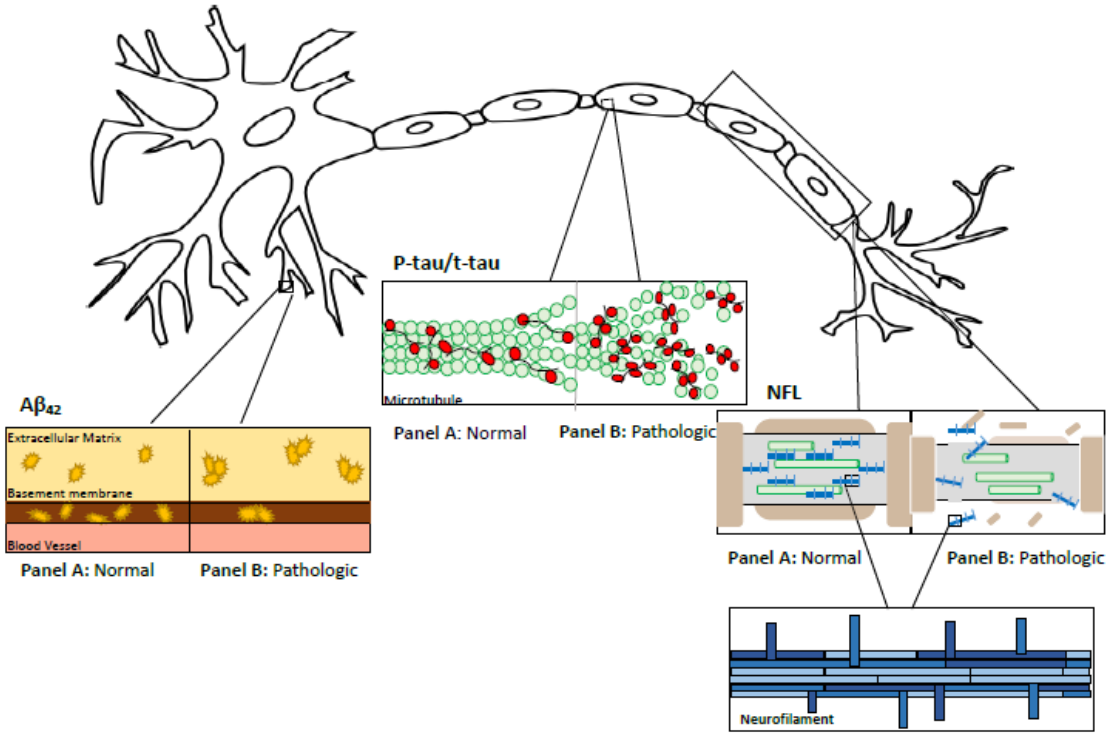


Figure 1 Caption. Depictions of pathophysiology associated with each CSF biomarker of interest: (1) $A\beta_{42}$ is a peptide made up of 42 amino acids, which results from cleavage of the amyloid precursor protein by beta and gamma secretase, forming larger peptide chains than its alpha secretase pathway. These larger peptides misfold into oligomers that form the amyloid plaques characteristic of AD. Panel A depicts effective clearance of smaller amyloid peptides through perivascular basement membranes. Panel B depicts impaired clearance of large Aβ peptides corresponding to increased amyloid deposition in the brain, resulting in Aβ oligomers in the extracellular matrix and lower concentrations of $A\beta_{42}$ in the CSF. (2) Tau is a protein found on microtubules that form the intracellular neurofibrillary tangles characteristic of AD. Higher CSF concentrations of tau occur in response to neurodegeneration. Hyperphosphorylated tau (p-tau) is measured by marking a specific phosphorylation site on the tau protein. P-tau measurements are more specific to Alzheimer's pathology than total tau due to the hyperphosphorylation of tau that occurs in AD. As depicted on Panel A, tau may be phosphorylated in its normal state and is involved in the dynamic instability of microtubules. Panel B illustrates how tau proteins become hyperphosphorylated in AD, causing tau to prematurely detach from microtubules and disrupt the balance of assembly and disassembly of microtubules. (3) Neurofilament light (NFL) is the smallest and most abundant of three polypeptides that form neurofilament proteins found in large-caliber, myelinated axons. As depicted in Panel A, neurofilaments exist in the axon alongside microtubules, increasing the axon's diameter and conduction velocity. When axonal damage occurs as illustrated in Panel B, neurofilaments spill into extracellular space and are cleared as cellular waste into the CSF. Higher CSF concentrations of NFL reflect the acute occurrence of axonal injury.

Figure 2. Participant Inclusion/Exclusion Details

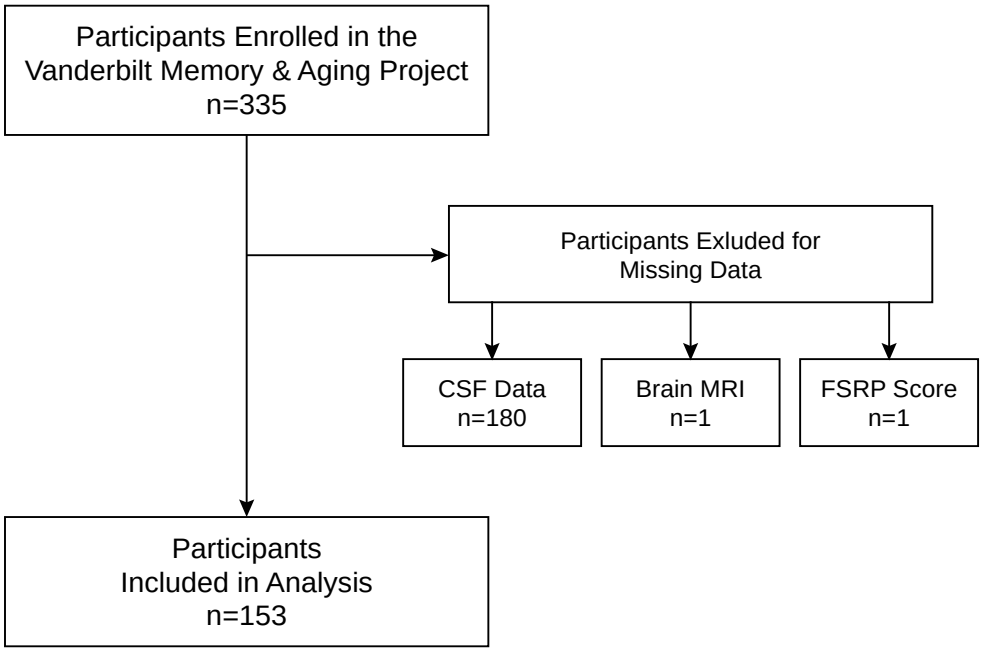
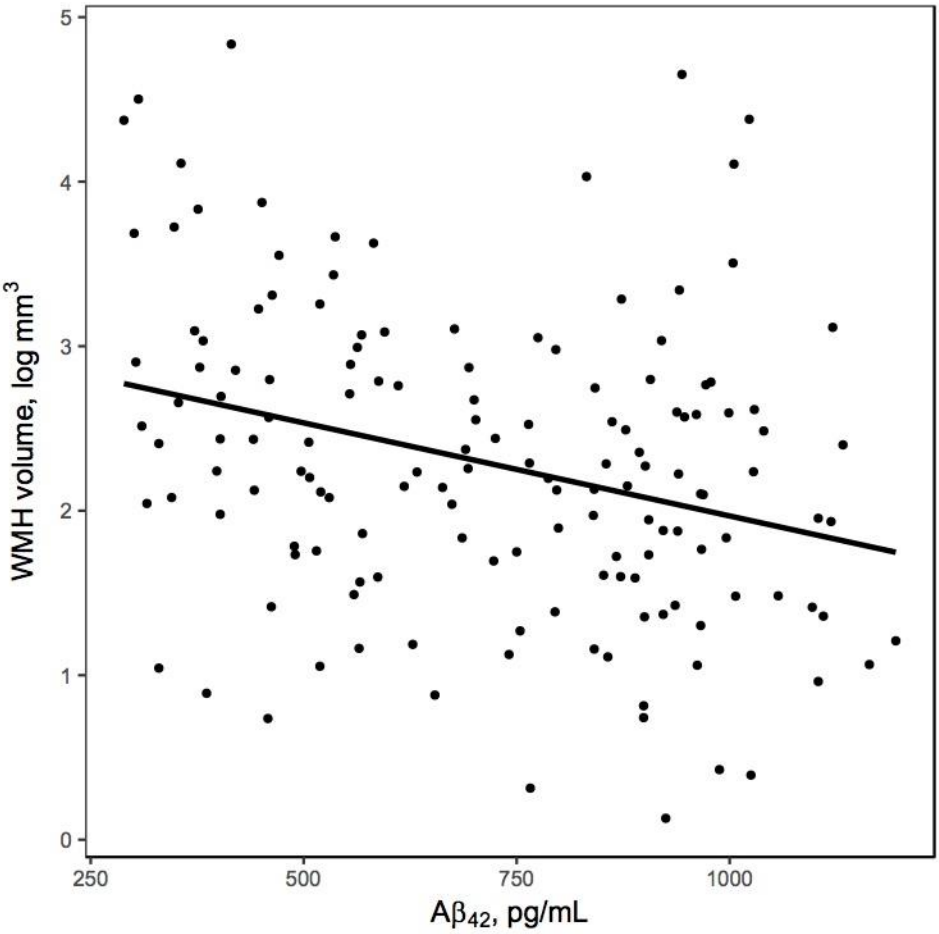


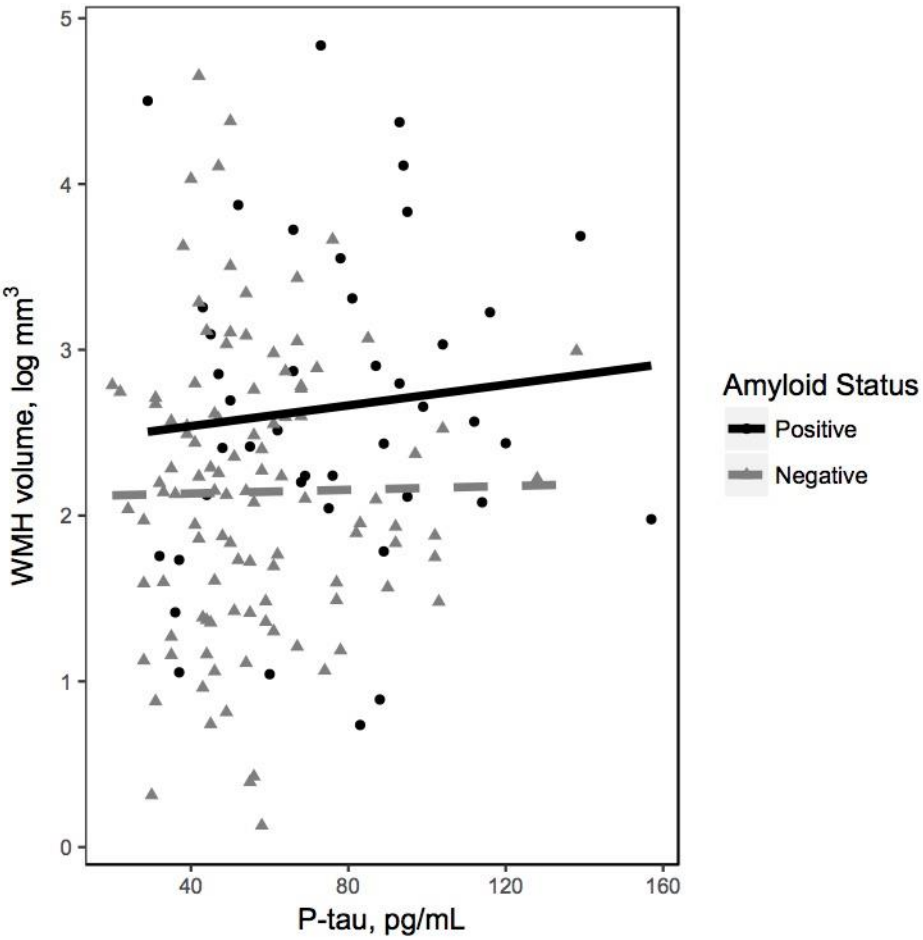
Figure 2 Caption. Missing data categories are mutually exclusive. CSF=cerebrospinal fluid; FSRP=Framingham Stroke Risk Profile

Figure 3. CSF Biomarker Concentrations & White Matter Hyperintensities

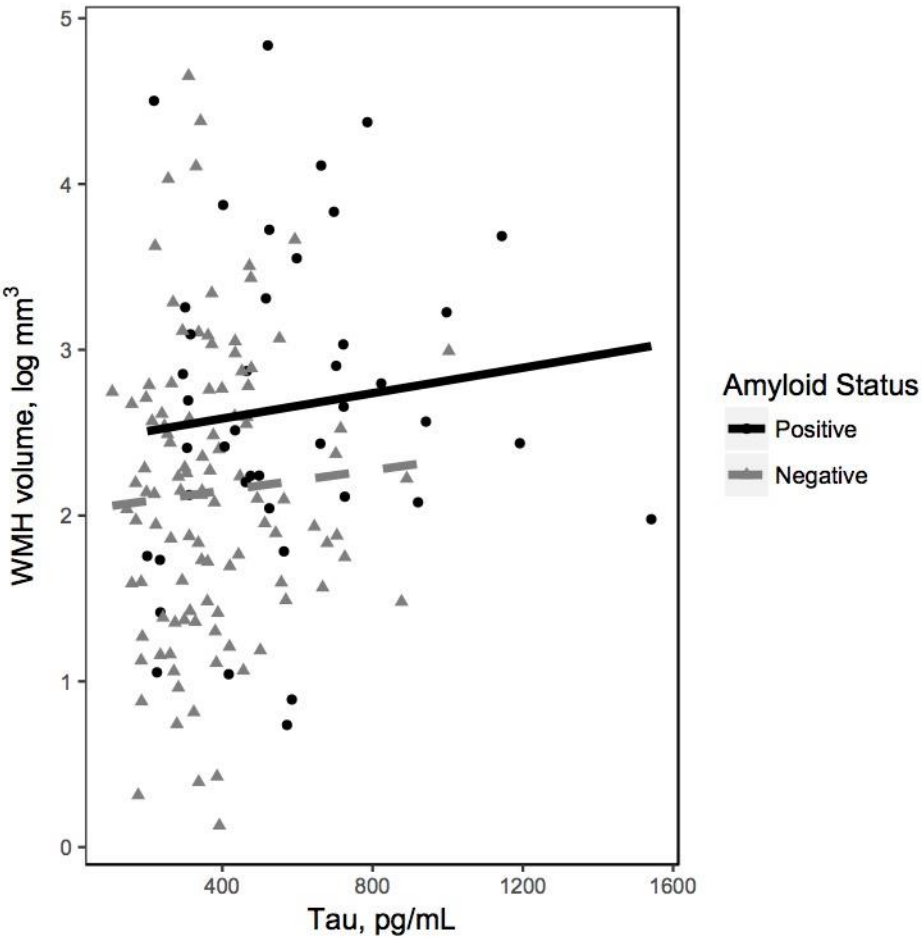
A. B-Amyloid$_{42}$ & White Matter Hyperintensities β=-0.001, p=0.003



B. Hyperphosphorylated Tau & White Matter Hyperintensities by Amyloid Status β=0.002, p=0.55



C. Total Tau & White Matter Hyperintensities by Amyloid Status β=0.0004, p=0.21



D. Neurofilament Light & White Matter Hyperintensities by Amyloid Status
β=0.0003, p=0.02

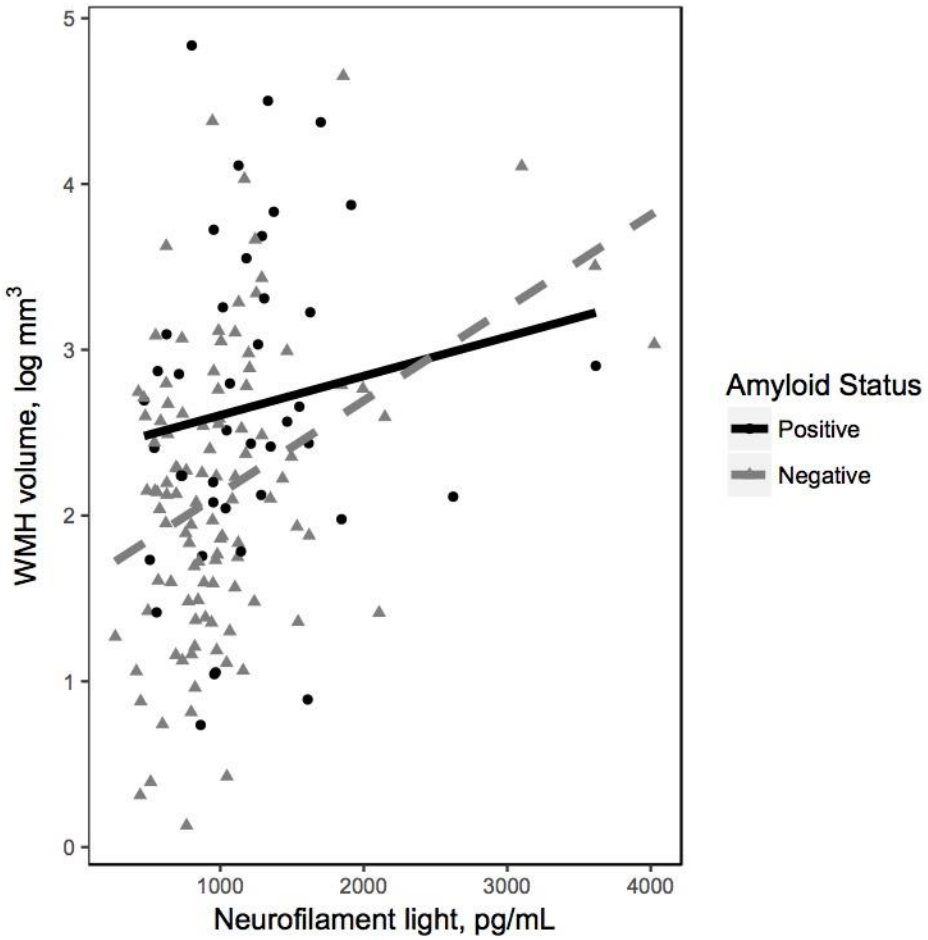


Figure 3 Caption. Solid black line reflects unadjusted values of WMH volume (Y axis) corresponding to CSF biomarker concentrations (X axis) , including (a) $A\beta_{42}$, (b) p-tau, (c) t-tau, and (d) NFL. WMH=white matter hyperintensities; $A\beta_{42}$=amyloid-β_{42}; P-tau=hyperphosphorylated tau; NFL=neurofilament light