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Regional White Matter Hyperintensities and Alzheimer's Disease Biomarkers Among Older Adults with Normal Cognition and Mild Cognitive Impairment

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Abstract.
Background: Alzheimer's disease (AD) frequently co-occurs with other brain pathologies. Recent studies suggest there may be a mechanistic link between AD and small vessel cerebrovascular disease (CVD), as opposed to simply the overlap of two disorders.
Objective: We investigated the cross-sectional relationship between white matter hyperintensity (WMH) volumes (markers of CVD) and cerebrospinal fluid (CSF) biomarkers of AD.
Methods: WMH volumes were assessed globally and regionally (i.e., frontal, parietal, temporal, occipital, and limbic). CSF AD biomarkers (i.e., $A\beta_{40}$, $A\beta_{42}$, $A\beta_{42}/A\beta_{40}$ ratio, phosphorylated tau-181 [p-tau_{181}], and total tau [t-tau]) were measured among 152 non-demented individuals (134 cognitively unimpaired and 18 with mild cognitive impairment (MCI)).
Results: Linear regression models showed that among all subjects, higher temporal WHM volumes were associated with AD biomarkers (higher levels of p-tau_{181}, t-tau, and $A\beta_{40}$), particularly among *APOE* ε4 carriers (independent of $A\beta_{42}$ levels). Higher vascular risk scores were associated with greater parietal and frontal WMH volumes (independent of CSF AD biomarker levels). Among subjects with MCI only, parietal WMH volumes were associated with a lower level of $A\beta_{42}/A\beta_{40}$. In addition, there was an association between higher global WMH volumes and higher CSF t-tau levels among younger participants versus older ones ($\sim$<65 versus 65+ years), independent of $A\beta_{42}/A\beta_{40}$ and p-tau_{181}.
Conclusion: These findings suggest that although WMH are primarily related to systemic vascular risk and neurodegeneration (i.e., t-tau), AD-specific pathways may contribute to the formation of WMH in a regionally-specific manner, with neurofibrillary tangles (i.e., p-tau) playing a role in temporal WMHs and amyloid (i.e., $A\beta_{42}/A\beta_{40}$) in parietal WMHs.

Keywords: Alzheimer's disease, amyloid, APOE, cerebrospinal fluid, magnetic resonance imaging, tau, vascular risk, white matter hyperintensity volumes

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INTRODUCTION

Alzheimer's disease (AD) pathology is present in a subset of older individuals who are cognitively normal [1]. Older adults without cognitive impairment also commonly have evidence of small vessel cerebrovascular disease (CVD) [2]. Whereas the pathological hallmarks of AD are plaques and tangles (primarily consisting of the accumulation of amyloid-β 42 ($A\beta_{42}$) and phosphorylated tau, respectively), the most common manifestation of CVD are white matter hyperintensities (WMH) on magnetic resonance imaging (MRI) scans [2]. WMH appear as bright, hyperintense regions in white matter on T2-weighted MR images. WMH are commonly thought to reflect demyelination and axonal loss resulting from chronic ischemia (i.e., reduced blood supply) due to the narrowing or occlusion of small vessels in the white matter. WMHs increase in prevalence with age, are associated with vascular risk factors [3], and contribute to cognitive decline and impairment [4, 5].

Although WMHs most commonly have a vascular origin [4], some evidence suggests that there may also be an AD-specific pathway contributing to WMH [6], particularly to WMH in the parietal lobe [7]. For example, pre-symptomatic carriers of autosomal dominant AD genetic mutations without appreciable vascular risk have elevated WMH volumes, in addition to abnormal AD biomarkers, several years before expected symptom onset, particularly in parietal and occipital regions [8, 9]. Similarly, prior studies have reported greater posterior WMHs (adjusting for global WMH burden) among participants with AD-dementia compared to older controls (e.g., [10, 11]). Additional evidence linking AD to ischemic damage comes from recent genetic and molecular work demonstrating that expression of many of the top AD risk genes is enriched in human brain vascular and perivascular cell types and that these cell types are selectively vulnerable to AD [12].

Studies examining the relationship between WMH and $A\beta_{42}$ among individuals with normal cognition have, however, been mixed. Supporting the view that there may be an AD-specific pathway contributing to WMH, several cross-sectional studies have reported associations between lower (i.e., more abnormal) levels of $A\beta_{42}$ in cerebrospinal fluid (CSF) and higher WMH volumes among older individuals with normal cognition [13–17]. By contrast, other studies have failed to find cross-sectional associations between CSF $A\beta_{42}$ levels and global WMH burden among older individuals with normal cognition [18–20] or subjective cognitive impairment [21]. Likewise, studies using amyloid PET imaging have found mixed results, with some studies observing cross-sectional associations between WMH load and amyloid among cognitively normal participants [22] and other studies not finding such relationships [23–26].

The first goal of the current study was to examine factors that might contribute to the difference in prior findings regarding the relationship between CSF $A\beta_{42}$ and WMH volumes among cognitively normal older adults, including participant characteristics that may have differed across studies (e.g., age, level of vascular risk and genetic risk for AD). In particular, we aimed to test the hypothesis that associations between $A\beta_{42}$ and WMH (both globally and regionally) may be stronger among carriers of the *APOE* ε4 allele, the major genetic risk factor for late-onset AD [27], than among ε4-non-carriers, as has been suggested by a few prior studies [17, but see 20, 28]. We also tested whether associations between WMH and CSF $A\beta_{42}$ are greater among participants with higher levels of vascular risk, based on reports that higher vascular risk may promote brain amyloid accumulation [29].

The second goal of the study was to examine whether CSF $A\beta_{42}$ levels are more strongly associated with WMH in specific subregions of the brain, rather than the brain as a whole, as there is little prior work on this topic among individuals with normal cognition [13]. A third goal of our study was to investigate the relationship between WMH and other AD-related CSF biomarkers (in addition to $A\beta_{42}$) that have received much less attention, particularly among participants with normal cognition. These include: 1) phosphorylated tau-181 (p-tau181), a marker of neurofibrillary tangle pathology, 2) total tau (t-tau), a general marker of neurodegeneration, 3) $A\beta_{40}$, the most abundant Aβ species, and 4) the ratio of $A\beta_{42}/A\beta_{40}$, which may be more specific to amyloid plaques than $A\beta_{42}$ alone [30, 31]. The two prior studies to examine the association between WMH and p-tau181 or t-tau among cognitively normal individuals found no significant relationships [17, 19], but associations with regional WMH volumes were not investigated. Likewise, only one study examined how CSF $A\beta_{40}$ relates to global WMH volumes among participants without cognitive impairment, finding a negative relationship between the two, but regional associations were not examined among the cognitively normal group [20]. Lastly, to our knowledge, $A\beta_{42}/A\beta_{40}$ has not been examined with respect to WMH among participants with normal cognition.

In order to examine these relationships between WMH burden and CSF AD biomarkers among older adults without cognitive impairment, we quantified both global and regional WMH volumes (frontal, parietal, occipital, temporal, and limbic) among 134 cognitively normal participants in relationship and CSF levels of $A\beta_{40}$, $A\beta_{42}$, $A\beta_{42}/A\beta_{40}$ ratio, p-tau181, and t-tau. Differences in patterns of associations by age, *APOE* $\varepsilon 4$ genotype, and level of vascular risk were systematically evaluated. Participants with mild cognitive impairment (MCI) (N = 18) were also included to investigate potential differences by diagnostic status.

MATERIALS AND METHODS

Study design and participant selection

This study makes use of data from participants in the ongoing Biomarkers for Older Controls at Risk for Dementia (BIOCARD) study. Established by the National Institute of Health (NIH) in 1995, the BIOCARD study is a longitudinal investigation of factors predicting the onset of symptoms of AD among individuals with normal cognition at baseline. Participants were recruited by staff members of Geriatric Psychiatry branch of the intramural program of the National Institute of Mental Health. By design, approximately 75% of the cohort has a first-degree relative with AD-dementia. Participants were excluded at baseline if they were cognitively impaired, had severe medical conditions, or reported substance abuse, resulting in a total enrollment of 349 cognitively normal and primarily middle-aged participants. Annual data collection consisted of a comprehensive battery of neuropsychological tests, clinical and neurological assessments, and blood draws; CSF samples and MRI scans were collected biennially. The study was halted in 2005 for administrative reasons and re-initiated at Johns Hopkins University (JHU) in 2009 to continue the annual clinical and cognitive evaluations, as well as blood draws. The biennial neuroimaging and CSF specimen collection resumed in 2015 at JHU. The neuroimaging protocol was expanded to include positron emission tomography (PET) scans using Pittsburgh Compound B (PiB PET) in 2015 and Tau PET in 2020. For additional information regarding participant recruitment and the clinical and cognitive assessments at baseline (see [32]).

The current study used cross-sectional data collected between 2015 and 2019 and included 152 non-demented older participants (mean age = 69.7) who provided both CSF and WMH MRI data at the same visit (i.e., within 2 days of one another). For participants with more than one set of matching CSF and MRI data during this 4-year time period (N = 110), the first available set of data was used. Among participants in the analysis, 134 were cognitively normal and 18 had a diagnosis of MCI. The study was approved by the JHU Institutional Review Board, and all participants provided written informed consent. Enrollment of additional participants was begun in 2020 and data collection for the entire cohort is ongoing.

Clinical and cognitive assessments

Clinical and cognitive assessments have been conducted annually since the study began. These assessments include a physical and neurological examination, record of medication use, mood and behavioral assessments, family history of dementia, history of symptom onset, a neuropsychological battery covering all major cognitive domains, and the Clinical Dementia Rating (CDR) based on a semi-structured interview [33].

Annual consensus diagnoses are conducted for each participant by the staff of the BIOCARD Clinical Core and follow the recommendations incorporated in the National Institute of Aging/Alzheimer's Association report for the diagnosis of MCI [34] and dementia due to AD [35]. First, a syndromic diagnosis is made (i.e., cognitively normal, MCI, impaired not MCI, or dementia) using three types of information: 1) decline in cognitive performance based on longitudinal cognitive test scores and comparison to published norms; 2) reports of changes in cognition by the individual and by collateral sources based on the CDR; and 3) clinical data regarding the medical, neurological, and psychiatric status of the individual. Second, if an individual is judged to be impaired, a decision about the likely etiology(ies) of the syndrome is made, using the psychiatric, neurological, and medical information. Individuals with contrasting information from the CDR interview and cognitive testing received a diagnosis of Impaired not MCI (e.g., the subject and/or collateral source reported concerns about cognition in daily life, but the cognitive testing did not show changes or vice versa). As in prior publications, participants with a diagnosis of Impaired not MCI ($n = 25$) were included in the group of cognitively normal subjects and all main analyses were re-run excluding these partici-

pants. Further details regarding the diagnostic process or neuropsychological battery can be found in Albert et al. [32]. The consensus diagnostic procedures are conducted without knowledge of the CSF and MRI data.

Summary vascular risk score

Vascular risk was assessed using a previously-validated summary score [29] based on the presence or absence of five vascular risk factors: hypertension, hypercholesterolemia, diabetes, obesity (defined as a body mass index greater than 30 kg/m^2), and smoking within the 30 days prior to data collection. This information was derived from participants' medical history reports or medical records collected at the same visit as the CSF and MRI data. The risk factors were coded dichotomously (0 if absent and 1 if present or remote) and then summed to calculate each individual's summary score (max = 5); for other studies using this score, see [29, 36–38].

APOE genotyping

APOE genotypes were determined by restriction endonuclease digestion of polymerase chain reaction amplified genomic DNA (performed by Athena Diagnostics, Worcester, MA). *APOE* $\varepsilon 4$ carrier status was dichotomized, with $\varepsilon 4$ carriers coded as 1 if they had at least one $\varepsilon 4$ allele and non-carriers coded as 0. Participants with $\varepsilon 2/\varepsilon 4$ alleles ($n = 2$) were included in the $\varepsilon 4$-carrier group, given that their risk for AD is similar to that of $\varepsilon 4$ carriers, rather than $\varepsilon 2$ carriers [39].

White matter hyperintensity volumes

MRI scans were acquired on a 3T Phillips Achieva scanner (Eindhoven, The Netherlands). The multi-modal protocol included magnetization-prepared rapid gradient echo (MPRAGE) scans used for anatomical reference and image registration (TR = 6.8 ms, TE = 3.1 ms, shot interval 3000 ms, flip angle = 8°, FOV = 240 × 256 mm^2, 170 slices with 1 × 1 × 1.2 mm^3 voxels, and scan duration = 5 min 59 s). White matter hyperintensity volumes were obtained from axial fluid-attenuated inversion recovery (FLAIR) MRI scans, which were acquired using a multi-slice fast spin-echo sequence (TR/TE/TI = 11000/100/2800 ms with 1 × 1 × 2 mm^3 resolution over an FOV of 256 × 256 × 138 mm^3; number of slices = 69; SENSE acceleration factor = 2.0; scan time = 3 min 18 sec).

Global WMH volumes were quantified using an automated method, the details of which are described in earlier publications [19, 40]. First, the skull was removed using an automatic atlas-based method [41]. The images were then nonlinearly registered by a cubic B-spline deformation to a minimal deformation template (MDT) synthetic brain image [42], adapted for ages 60 years and older. Second, a template-based iterative method was used for correcting field inhomogeneity bias [43]. The bias field was modeled using a spatially smooth thin-plate spline interpolation based on ratios of local image patch intensity means between the deformed template and subject images. Third, to segment gray, white, and CSF tissues, we used an Expectation-Maximization algorithm, which generates segmentations that are most consistent with the input intensities from the native-space T1 images along with a model of image smoothness using an iterative process [44]. Fourth, WMH measures were calculated based on a combination of FLAIR and 3D T1 images using a modified Bayesian probability structure utilizing histogram fitting [45]. Initially, all segmentation was performed in standard space, which produced probability likelihood values of WMH at each voxel in the white matter. We then thresholded these probabilities using a cutoff of 3.5 SDs above the mean to create a binary WMH mask. Further segmentation involved a modified Bayesian approach, in which image likelihood estimates are combined with spatial priors and with constraints on tissue class. For the calculation of tissue volumes, the segmented WMH masks were back-transformed to native space. All images were visually inspected for gross motion artifacts prior to running the pipeline and none were detected.

In addition, WMH volumes were quantified within the following regions: frontal, parietal, temporal, occipital, and limbic. To compute region-specific WMH volumes, the T1-MRPAGE image was first registered to FLAIR space and segmented with MRICloud to obtain individual masks for each of the 5 regions. MRICloud is a public web-based service for multi-contrast image segmentation and quantification (https://www.MRICloud.org, Johns Hopkins University, MD). Then the regional masks were combined with the global WMH mask to quantify the regional WMH volumes. See Fig. 1 for an example of the segmented WMHs and the 5 regions examined. Global and regional WMH volumes were log-transformed for all regression analyses to correct for skewness.

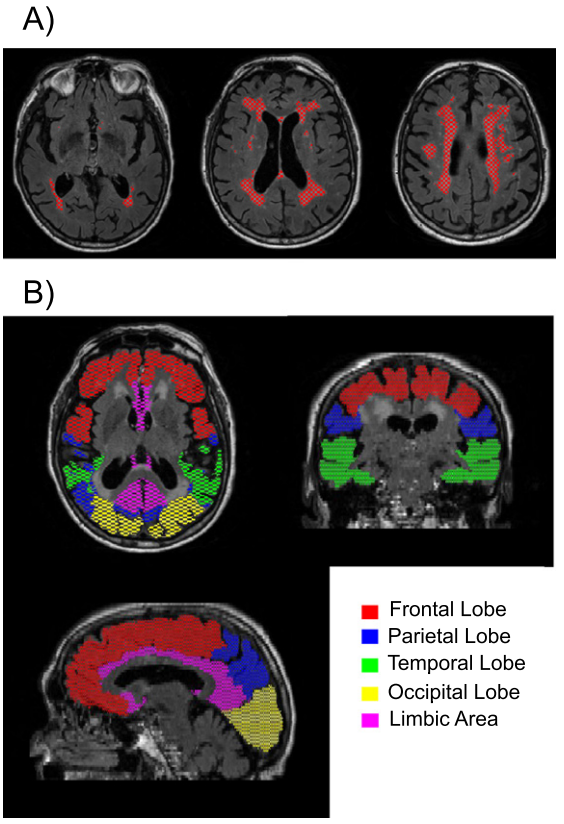

Fig. 1. Example of A) segmented white matter hyperintensities are shown in red on three different axial slices; and B) the five regions of interest examined are shown in different colors.

WMH volumes were not adjusted for total intracranial volume (TICV) because TICV was not correlated with global WMH volume ($r = 0.02$ using the raw global WMH volumes and $r = 0.08$ using the log-transformed global WMH volumes).

CSF assessments

CSF (20 ml) was collected via lumbar puncture into 50 ml polypropylene tubes in the morning after overnight fasting. After mixing and centrifugation at 2000 rpm for 15 min, 500 µl aliquots of CSF were frozen at –80°C within 60 min of collection. The CSF samples were analyzed using the fully automated electrochemiluminescence enzyme immunosassays (Lumipulse G1200) for $A\beta_{40}$, $A\beta_{42}$, pTau181, and total Tau (tTau). Assays were run in duplicate, and all samples were run in a single batch. The coefficients of variation (CV) of an internal CSF standard run every day over the 10 days the assays were performed were: $A\beta_{1\text{-}42}$, 3.4%; $A\beta_{1\text{-}40}$, 2.7%; ptau181, 1.8% and t-tau, 8.0%. In addition, the ratio of $A\beta_{42}/A\beta_{40}$ was calculated because previous reports indicate that it accounts for inter-individual differences in total CSF Aβ levels and may mitigate against pre-analytic variability [30, 31]. The CSF measures were log-transformed for all analyses to correct for skewness.

Statistical analysis

We compared group differences in descriptive statistics using two-tailed *t*-tests for continuous variables and with chi-square tests for categorical variables. The first set of analyses examined the relationship between the 5 CSF biomarkers (i.e., $A\beta_{40}$, $A\beta_{42}$, pTau181, and tTau, and $A\beta_{42}/A\beta_{40}$) and global WMH volume, using linear regression models, uncorrected for multiple comparisons. All analyses used separate linear regression models for each CSF measure, with WMH volumes as dependent variables and the CSF biomarkers as independent variables, including age, sex, years of education, and diagnostic status (coded dichotomously: cognitively normal = 0 versus MCI = 1) as covariates. Follow-up linear regression models then explored whether the relationship between the 5 CSF measures and global WMH volume differed by age, diagnostic status, *APOE* ε4 status, or vascular risk score by including these variables and their interactions (i.e., cross-products) with the CSF measure as predictors (covarying sex and education). All interaction terms were included in the same model (i.e., age x CSF; diagnostic status x CSF, *APOE* ε4 × CSF, and vascular risk score x CSF) to reduce the number of models run.

The second set of analyses investigated the associations between each CSF biomarker and the 5 regional WMH volumes, also in separate linear regressions, using a Bonferroni correction for multiple comparison (i.e., 5 tests, with p values <0.01 considered significant). For the regional WMH analyses, significant p-values using a Bonferroni correction for 25 tests (i.e., 5 CSF × 5 WMH variables) are also reported. As for the analyses examining global WMH volume, follow-up exploratory linear regression analyses were conducted to determine whether age, diagnostic status, *APOE* ε4 genetic status, or the summary vascular risk score modified the associations between the CSF and WMH variables by including interaction terms of CSF x age/ diagnostic status/ *APOE* ε4/ vascular risk score in the same model. When significant interaction terms were present, stratified follow-up models were run to examine associations separately in relevant subgroups.

Two sets of sensitivity analyses were conducted. First, we tested whether the main results remained the same in the subgroup of participants with normal cognition and when participants with a diagnosis of Impaired-not-MCI were excluded. Second, given the potential importance of hypertension in WMH development, we re-ran the analyses using the vascular risk summary score, instead using 1) an indicator for hypertension (present versus absent) and 2) a modified vascular risk score based only on obesity, smoking, hypercholesterolemia, and diabetes (min = 0, max = 4). In these sensitivity analyses, both the hypertension indicator and the modified vascular summary score were simultaneously included as predictors. All analyses were run in R (version 1.4.1106) and SAS 9.4.

RESULTS

Table 1 details the characteristics of participants stratified by diagnosis. Participants diagnosed with MCI had higher vascular risk scores, on average ($t(151) = 2.02$, $p = 0.046$), as well as greater limbic WMH volumes ($t(151) = 2.59$, $p = 0.01$) than the cognitively normal group. The difference in limbic WMH burden between the MCI and cognitively normal group remained significant when adjusting for age, sex, and education and when using the log-transformed WMH instead of the raw values ($t = 2.55$, $p = 0.012$), while the group difference in vascular risk scores was no longer significant after covariate adjustment ($t = 1.75$, $p = 0.082$). The correlations among the 5 regional log-transformed WMH volumes ranged from $r = 0.27$ to 0.66, all $p < 0.001$, indicating moderate correlations.

Associations between CSF measures and global WMH volume

Results from the linear regression analyses examining global WMH volumes among all non-demented participants indicated no significant associations with any of the CSF measures (all $p > 0.12$ uncorrected). However, there was a strong association between older age and higher global WMH volumes ($p < 0.0001$ in all models), while sex (all $p > 0.49$) and years of education (all $p > 0.67$) were unrelated to global WMH volume in all models. Results were the same in the subgroup of individuals with normal cognition, demonstrating no significant associations between the CSF measures and global WMH volume.

Analyses examining potential modifiers of the relationship between the CSF measures and global WMH volumes found no interactions between the CSF measures and diagnostic status (all $p > 0.54$), *APOE* ε4 status (all $p > 0.48$), and the vascular risk score (all $p > 0.53$) in relationship to global WMH volume. Additionally, the vascular risk score and *APOE* ε4 genetic status were not associated with global WMH volume (both $p > 0.26$). However, there was a significant interaction between age and t-tau (*estimate* = –0.06, $SE = 0.03$, $p = 0.024$) in relationship to global WMH volume, such that as participants' age decreased, the association between t-tau and global WMH volume increased. For example, among participants aged 65 years and under, higher CSF t-tau was associated with greater global WMH volumes ($n = 38$, *estimate* = 1.24, $SE = 0.56$, $p = 0.034$), whereas this association was not significant among participants over age 65 ($n = 115$, *estimate* = –0.09, $SE = 0.22$, $p = 0.68$). This is illustrated in Fig. 2. The interaction between age and t-tau was significant in the cognitively normal subgroup (*estimate* = –0.08, $SE = 0.03$, $p = 0.006$). It remained significant when additionally adjusting for $A\beta_{42}/A\beta_{40}$ and *p*-tau181 in the same model (total sample *estimate* = –0.06, $SE = 0.02$, $p = 0.014$; cognitively normal sample *estimate* = –0.08, $SE = 0.03$, $p = 0.006$). The pattern of results was the same when using total subcortical WMH volumes, as when using total WMH volumes (data not shown).

Although interactions between the CSF measures and *APOE* ε4 genetic status were not significant in relationship to global WMH volumes, stratified analyses were run by *APOE* ε4 genetic status, given prior reports of differences [17, 28]. These showed that lower levels of $A\beta_{42}/A\beta_{40}$ were associated with higher global WMH volumes among *APOE* ε4 carriers (total sample $p = 0.036$; cognitively normal sample $p = 0.051$) but not non-carriers ($p > 0.4$).

Associations between CSF measures and regional WMH volumes

The regional analyses revealed that among all participants, higher temporal WMH volumes were associated with significantly higher levels of $A\beta_{40}$, p-tau181, and t-tau (all $p < 0.007$, see Table 2 for full model results). Scatterplots illustrating these relationships are shown in Fig. 3. There were no significant associations between the CSF measures and WMH volumes in any of the other regions (see Table 2). Results were the same in the cognitively normal sub-

Table 1
Participant characteristics for total sample and stratified by diagnosis

Variable	Total Sample (N = 152)	Cognitively Normal (N = 134)	MCI (N = 18)
Age, y, mean (SD)	69.3 (8.5)	69.1 (8.6)	69.1 (7.9)
Sex, number of females (%)	97 (63.8%)	87 (64.9%)	10 (55.6%)
Education, y, mean (SD)	17.1 (2.4)	17.3 (2.3)	16.4 (2.7)*
MMSE score, mean (SD)	28.9 (1.5)	29.2 (1.0)	27.4 (1.6)***
APOE ε4 carriers, number (%)	52 (34.2%)	46 (34.3%)	6 (33.3)
Vascular Risk Score, mean (SD)	1.4 (1.0)	1.3 (1.0)	1.8 (1.1)*
Vascular Risk Score ≥1, number (%)	115 (75.7%)	101 (75.4%)	13 (72.2%)
Vascular Risk Score ≥2, number (%)	63 (41.5%)	53 (39.6%)	9 (50%.0)
Global WMH volume (mm^3), mean (SD)	4281 (6931)	4302 (7290)	4214 (3292)
Frontal WMH volume (mm^3), mean (SD)	118 (426)	127 (453)	45 (97)
Parietal WMH volume (mm^3), mean (SD)	70(19)	74 (200)	35 (109)
Temporal WMH volume (mm^3), mean (SD)	21 (68)	23 (72)	10 (12)
Occipital WMH volume (mm^3), mean (SD)	267 (462)	260 (478)	320 (321)
Limbic WMH volume (mm^3), mean (SD)	177 (258)	151 (207)	369 (462)*
CSF $A\beta_{40}$ (pg/ml), mean (SD)	11968 (4142)	12099 (4180)	10995 (3819)
CSF $A\beta_{42}$ (pg/ml), mean (SD)	961 (464)	966 (463)	924 (486)
CSF $A\beta_{42}/A\beta_{40}$, mean (SD)	0.08 (0.02)	0.08 (0.02)	0.08 (0.03)
CSF p-tau181 (pg/ml), mean (SD)	42 (21)	42 (21)	38 (19)
CSF t-tau (pg/ml), mean (SD)	301 (149)	305 (152)	273 (124)

*$p < 0.05$; **$p < 0.005$; ***$p < 0.0005$ indicate significant differences between the groups of cognitively normal and MCI participants.

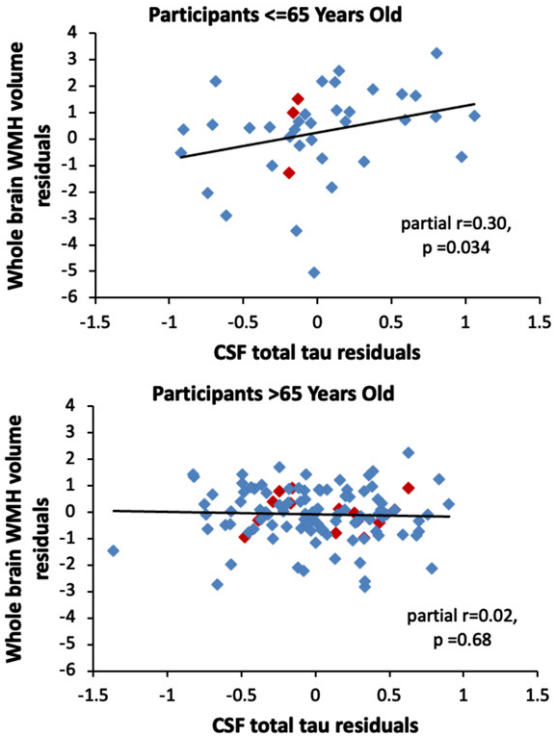

Fig. 2. Scatterplots showing the partial correlations between global white matter hyperintensity volumes (y-axis) and CSF total tau levels (x-axis) for participants aged 65 years or younger (top panel) and participants older than 65 years (bottom panel). Correlations are adjusted for age, sex, years of education, diagnostic status, *APOE* ε4 genetic status, and summary vascular risk scores. Participants with a diagnosis of MCI are shown red (◇); participants with normal cognition are shown in blue (◇).

group (see Table 2 for model results). Results also remained the same when additionally adjusting for *APOE* ε4 genotype and the summary vascular risk score, with higher temporal WMH volumes being associated with higher p-tau181 (estimate = 0.92, SE = 0.28, $p = 0.0014$) and t-tau (*estimate* = 1.03, *SE* = 0.27, $p = 0.0002$), and $A\beta_{40}$ (*estimate* = 0.95, *SE* = 0.36, $p = 0.0086$). In this fully-adjusted model, the associations of temporal WMH with p-tau181 and t-tau were significant at the more stringent correction for multiple comparisons ($p < 0.002$), while the association with $A\beta_{40}$ was not significant at this threshold. The association between temporal WMH volume and t-tau or p-tau also remained significant when additionally adjusting for CSF $A\beta_{42}$ or $A\beta_{42}/A\beta_{40}$ (all $p \leq 0.004$). All results were unchanged in the cognitively normal subgroup (adjusting for *APOE* ε4, vascular risk score, and $A\beta_{42}/A\beta_{40}$, tau *estimate* = 1.06, *SE* = 0.30, $p = 0.0005$; p-tau *estimate* = 0.93, *SE* = 0.32, $p = 0.0038$).

A few recent studies have suggested that among participants with normal cognition, it may be helpful to correct measures of t-tau and p-tau for individual differences in CSF production and drainage rates by using the ratios of t-tau/$A\beta_{40}$ or p-tau/$A\beta_{40}$ [46, 47]. In a *post-hoc* analysis including only individuals with normal cognition, adjusting for age, sex, education, *APOE* ε4 genotype, vascular risk score, and $A\beta_{42}$, higher ratios of t-tau/$A\beta_{40}$ (*estimate* = 1.68, *SE* = 0.50, $p = 0.0011$) and p-tau181/$A\beta_{40}$ (*estimate* = 1.35, *SE* = 0.54, $p = 0.014$)

Table 2
Linear regression results for associations between regional WMH burden and CSF AD biomarker levels

WMH Region	$A\beta_{40}$		$A\beta_{42}$		$A\beta_{42/40}$		pTau181		tTau	
	estimate (SE)	p	estimate (SE)	p	estimate (SE)	p	estimate (SE)	p	estimate (SE)	p
All Non-Demented Participants (N = 152)										
Frontal	–0.05 (0.52)	n.s.	–0.01 (0.37)	n.s.	–0.14 (4.78)	n.s	0.30 (0.40)	n.s.	0.21 (0.40)	n.s.
Parietal	0.33 (0.50)	n.s.	–0.01 (0.36)	n.s.	–2.28 (4.61)	n.s.	0.49 (0.38)	n.s.	0.36 (0.38)	n.s.
Temporal	0.98 (0.36)	0.0066	0.44 (0.26)	n.s.	1.28 (3.36)	n.s.	0.79 (0.27)	0.004	0.97 (0.27)	0.0004*
Occipital	0.58 (0.40)	n.s.	–0.20 (0.29)	n.s.	–7.33 (3.64)	n.s.	0.63 (0.30)	n.s.	0.47 (0.31)	n.s.
Limbic	–0.58 (0.36)	n.s.	–0.54 (0.26)	n.s.	–5.78 (3.33)	n.s.	–0.13 (0.28)	n.s.	–0.20 (0.28)	n.s.
Cognitively Normal Participants (N = 134)										
Frontal	–0.07 (0.57)	n.s.	0.16 (0.41)	n.s.	3.17 (5.19)	n.s	0.03 (0.43)	n.s.	0.05 (0.42)	n.s.
Parietal	0.41 (0.54)	n.s.	0.29 (0.39)	n.s.	2.23 (4.94)	n.s.	0.21 (0.40)	n.s.	0.14 (0.40)	n.s.
Temporal	0.96 (0.39)	0.0066	0.41 (0.29)	n.s.	0.94 (3.68)	n.s.	0.78 (0.29)	0.0080	0.98 (0.29)	0.0008*
Occipital	0.84 (0.42)	n.s.	0.01 (0.31)	n.s.	–5.29 (3.88)	n.s.	0.68 (0.31)	n.s.	0.57 (0.31)	n.s.
Limbic	–0.49 (0.39)	n.s.	–0.47 (0.28)	n.s.	–4.87 (3.59)	n.s.	–0.15 (0.30)	n.s.	–0.01 (0.29)	n.s.
MCI (N = 18)										
Parietal	–0.40 (1.32)	n.s.	–1.83 (0.79)	0.037	–36.15 (8.87)	0.0013	3.00 (1.08)	0.015	2.69 (1.34)	n.s.

The table shows *p*-values significant after Bonferroni adjustment for 5 comparisons (i.e., $p < 0.01$). *p*-values >0.01 are reported as not significant (n.s.). *indicates *p*-values significant after Bonferroni adjustment for 25 comparisons (i.e., $p < 0.002$).

were associated with higher temporal WMH volumes.

CSF measures and regional WMH volumes: The role of diagnostic status

For parietal WMH volumes only, there was an interaction between diagnostic status and $A\beta_{42}/A\beta_{40}$ (*estimate* = –33.87, *SE* = 12.66, $p = 0.009$). Follow-up models, stratified by diagnostic status, revealed that among participants with MCI, lower $A\beta_{42}/A\beta_{40}$ (*estimate* = –31.48, *SE* = 12.01, $p = 0.02$) was associated with greater parietal WMH volumes, but this association was not significant among those with normal cognition ($p > 0.4$; see Table 2). The interaction between diagnostic status and $A\beta_{42}$ only approached significance ($p = 0.08$).

CSF measures and regional WMH volumes: The role of vascular risk

There were no significant interactions between the summary vascular risk score and any of the CSF measures with respect to the regional WMH variables in the full sample (all $p > 0.07$) or in the cognitively normal subgroup (all $p > 0.07$), suggesting that the relationship between the CSF measures and regional WMH volumes do not differ by level of vascular risk. However, higher vascular risk summary scores were associated with higher parietal WMH volumes (*estimate* = 0.37, *SE* = 0.17, $p = 0.028$) and marginally higher frontal WMH volumes (*estimate* = 0.33, *SE* = 0.17, $p = 0.056$), while associations with other WMH regions were not significant (all $p > 0.2$) (covarying age, sex, education, and diagnostic status). These results were the same in the cognitively normal subgroup (parietal *estimate* = 0.39, *SE* = 0.18, $p = 0.03$; frontal *estimate* = 0.37, *SE* = 0.19, $p = 0.054$). Higher vascular risk summary scores remained associated with higher parietal WMH volumes when also adjusting for *APOE* ε4 genetic status, as well as for CSF $A\beta_{42}/A\beta_{40}$, p-tau181, and t-tau (total sample *estimate* = 0.36, *SE* = 0.17, $p = 0.03$; cognitively normal sample *estimate* = 0.37, *SE* = 0.18, $p = 0.04$). This suggests that the relationship between the vascular risk score and parietal WMH volumes is independent of AD-biomarker levels (Fig. 4 portrays visually the brain regions where there was an association between WMH and vascular risk compared to the regional association of WMH and biomarkers of tau and p-tau, mentioned above).

CSF measures and regional WMH volumes: The role of APOE ε4 genetic status

There were no significant interactions between *APOE* ε4 status or age and any of the CSF measures with respect to the regional WMH measures in the full non-demented sample (all $p > 0.07$) or in the cognitively normal subgroup (all $p > 0.12$). However, because some previous studies suggest that associations between global WMH volume and AD biomarker levels may be greater among *APOE* ε4 carriers than non-carriers [17, 28], *post-hoc* analyses stratified by ε4 carrier status were also run for tem-

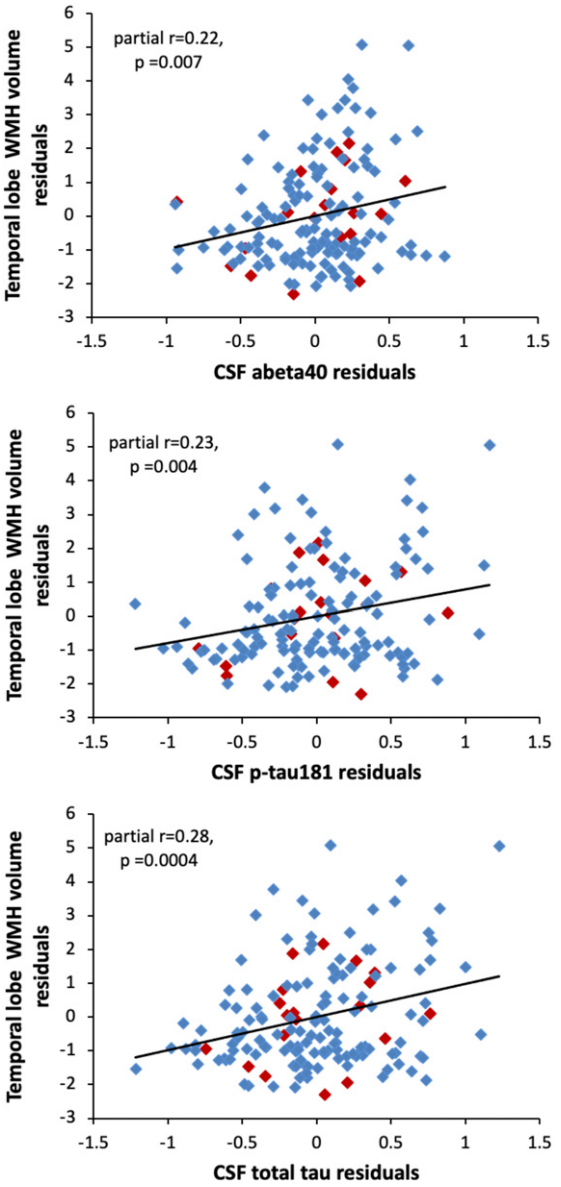

Fig. 3. Scatterplots showing the partial correlations between white matter hyperintensity volumes in the temporal lobe (y-axis) and CSF levels of $A\beta_{40}$ (top panel), p-tau181 (middle panel), and t-tau (bottom panel). Correlations are adjusted for age, sex, years of education, and diagnostic status. Participants with a diagnosis of MCI are shown red (◇); participants with normal cognition are shown in blue (◇).

poral WMH volumes, for which there were robust associations with CSF p-tau, t-tau, and $A\beta_{40}$ (see above). This revealed that the relationship between temporal WMH volumes and CSF $A\beta_{40}$, p-tau181, and t-tau measures were significant among *APOE* ε4 allele carriers ($n = 52$, all $p < 0.013$, see Table 3 for

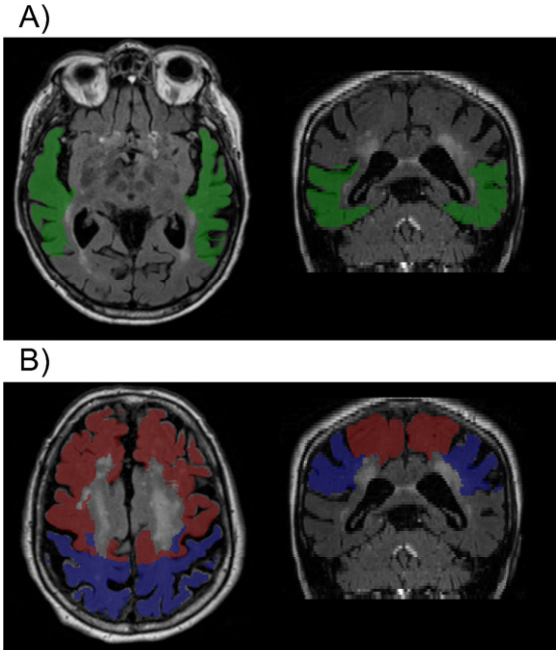

Fig. 4. Example of segmentation showing A) the temporal region (green) where higher WMH volumes were associated with higher levels of p-tau and t-tau; and B) parietal (blue) and frontal (red) regions, where greater WMH volumes where associated with higher summary vascular risk scores (see text for details).

full model results), but not in non-carriers ($n = 100$, all $p > 0.09$).

Additional analyses showed that the vascular risk score did not differ between *APOE* ε4 carriers and non-carriers (adjusting for age, sex, and education, and diagnostic status, *estimate* = –0.15, $SE = 0.18$, $p = 0.41$). This suggests that differential associations between WMH and CSF biomarkers by *APOE* ε4 genetic status does not reflect differences in levels of vascular risk between ε4 carriers and non-carriers. *APOE* ε4 carriers and non-carriers did not differ in terms of global or regional WMH volumes (all $p > 0.23$). The differences between *APOE* ε4 carriers and non-carriers were the same in the cognitively normal subgroup (data not shown). However, as would be expected, *APOE* ε4 carriers had lower levels of CSF $A\beta_{42}$ (*estimate* = –0.47, $SE = 0.07$, $p < 0.0001$) and $A\beta_{42}/A\beta_{40}$ (*estimate* = –0.04, $SE = 0.005$, $p < 0.0001$), and higher levels of p-tau181 (*estimate* = 0.29, $SE = 0.08$, $p = 0.0002$) and t-tau (*estimate* = 0.20, $SE = 0.08$, $p = 0.012$).

Table 3
Linear regression results for associations between temporal WMH and CSF AD biomarker levels for *APOE* ε4 carriers and non-carriers

colrule Diagnostic Group	Aβ40		pTau181		tTau	
	Estimate (SE)	*p*	Estimate (SE)	*p*	Estimate (SE)	*p*
APOE ε4 carriers						
Non-demented($n=52$)	1.47 (0.56)	0.0125	1.33 (0.37)	0.0009*	1.42 (0.37)	0.0003*
Cognitively normal ($n=46$)	1.42 (0.60)	0.0234	1.33 (0.41)	0.0022	1.42 (0.39)	0.0008*
APOE ε4 non-carriers						
Non-demented($n=100$)	0.66 (0.47)	n.s.	0.52 (0.41)	n.s.	0.69 (0.39)	n.s.
Cognitively normal ($n=88$)	0.61 (0.53)	n.s.	0.45 (0.44)	n.s.	0.69 (0.42)	n.s.

CSF measures and regional WMH volumes: The role of participant age

Associations between the CSF measures and regional WMH volumes did not differ by participant age (all $p > 0.07$).

Sensitivity analyses

The pattern of results was the same when participants with a diagnosis of Impaired not MCI were excluded from the analysis (data not shown). In addition, consistent with the results using the vascular risk summary score, neither the indicator for hypertension, nor the modified vascular risk score, altered the associations between the CSF measures and the global or regional WMH volumes in the total sample or in the cognitively normal subgroup (for all interactions, $p > 0.09$). However, higher parietal ($estimate = 0.78$, $SE = 0.38$, $p = 0.037$) and frontal ($estimate = 0.76$, $SE = 0.39$, $p = 0.055$) WMH volumes were associated with hypertension, but not with the modified vascular risk score (both $p > 0.4$), underscoring the importance of hypertension for the development of region-specific WMH.

DISCUSSION

This study examined the cross-sectional association between global and regional WMH volumes and CSF biomarkers of amyloid, total tau, and phosphorylated tau among participants with normal cognition and MCI. There are several notable findings. First, there was an association between higher global WMH volumes and higher CSF t-tau levels among participants with lower baseline ages (∼<65 years), but not those with higher baseline ages. This association was independent of AD CSF biomarker levels (i.e., $A\beta_{42/40}$ and p-tau181), suggesting the link between global WMH burden and levels of neuronal injury may be unrelated to amyloid and neurofibrillary tangle pathology. A second major finding was that higher temporal WMH volumes were related to higher CSF levels of $A\beta_{40}$, p-tau181, and t-tau in the total sample and in the cognitively normal subgroup, and that this relationship was particularly evident among *APOE* ε4 carriers. These associations were also independent of CSF $A\beta_{42}$ levels, suggesting they may also be unrelated to plaque deposition. Lastly, higher parietal WMH volumes were associated with lower (i.e., more abnormal) levels of $A\beta_{42/40}$ among participants with MCI only, and with higher vascular risk scores (particularly hypertension) among all participants. Taken together, these results support the view that among participants with normal cognition, WMH are primarily associated with vascular and tau-related neurodegenerative factors, while among participants with MCI, WMH may also be related to amyloidosis. Our results also suggest that among individuals with normal cognition (and relatively low levels of AD pathology) vascular risk promotes WMH accumulation independently of AD pathology levels, whereas among cognitively impaired individuals (with higher levels of AD pathology), both vascular risk and amyloidosis contribute to greater WMH burden.

Temporal WMH volumes and CSF AD biomarkers

The finding that higher temporal WMH volumes are related to higher p-tau181 and t-tau levels in CSF, independent of $A\beta_{42}$ or $A\beta_{42}/A\beta_{40}$ levels, may indicate that WMH are associated with neurodegeneration resulting from both neurofibrillary-tangle related pathology as well as neuronal damage from other causes [48]. This interpretation would be consistent with the finding that neurofibrillary tangles first accumulate in the medial temporal lobe before spreading to other cortical regions [49]. Among older individuals with normal cognition, neurofibrillary tangle load and related axonal damage would therefore be expected to be highest in temporal regions.

This would explain why the association of p-tau and t-tau was significant for temporal WMH volumes, but not other brain regions, nor with global WMH burden.

Importantly, these associations appear to be stronger among *APOE* ε4 carriers than non-carriers, suggestive of an AD-specific pathway. It has also been suggested that *APOE* ε4 may have effects on tau via Aβ-independent mechanisms [50]. Another possibility is that small cerebral vessels in the temporal lobe are particularly sensitive to the effects of systemic vascular risk factors or to other *APOE* ε4 related mechanisms. In this view, chronic hypoperfusion and resulting ischemia of temporal white matter regions would result in the demyelination of axons, axonal degeneration, and ultimately tangle formation [51]. This would be consistent with recent neuropathological findings that higher tangle pathology is associated with both greater small-vessel disease burden in posterior brain regions (measured by arteriosclerosis [52]) and with higher global WMH among older adults across the clinical spectrum of AD [52, 53]. Further supporting this view, a mouse model of ischemia demonstrated that hypoperfusion can promote higher phosphorylated and total tau accumulation [53].

Our findings are in line with, and extend, prior studies among participants with MCI and across the spectrum of AD showing that higher global WMH load is associated with higher levels of CSF t-tau [21], greater increases in t-tau over time [54], higher p-tau181/$A\beta_{40}$ ratios [46], as well as with higher plasma tau levels [53]. Our results are also consistent with the finding that higher Braak scores, which are indicative of higher tangle-related pathology, are associated with higher levels of WMH [53, 55] among individuals across the AD-spectrum. Of note, several prior studies did not find associations between global WMH volumes and CSF levels of p-tau or t-tau among cognitively normal [17, 19] and non-demented samples [8, 13, 16, 56], but temporal lobe WMH were not investigated in these studies. The current results emphasize the importance of investigating regional WMH, as relationships with AD biomarkers may vary across the brain and over the course of the disease.

Notably, in the current study, higher $A\beta_{40}$ levels were also associated with higher temporal WMH volumes among all subjects and the cognitively normal subgroup, though this association was less robust than for p-tau and t-tau. Although altered levels of $A\beta_{40}$ are not typically observed in AD, including the preclinical phase (e.g., [57]), a recent multicenter study reported slightly, but significantly higher levels of $A\beta_{40}$ among patients with AD-dementia compared to controls [58]. Within this context, it has been suggested that increased $A\beta_{40}$ may be a marker of elevated overall amyloid production [46, 58], overall CSF production [59], or of reduced clearance of peptides from the brain into the CSF [47]. For these reasons, $A\beta_{40}$ is often used to correct for pre-analytic variables that affect $A\beta_{42}$ [60]. The current results therefore suggest that higher total amyloid production or higher overall CSF production or reduced CSF drainage among cognitively normal older adults is associated with greater temporal WMH volumes.

Global WMH volumes and CSF total tau

We also observed an age-dependent relationship between higher global WMH and higher t-tau levels that was significant for participants with lower baseline ages (∼<65 years) but not those with higher baseline ages. This association remained when adjusting for $A\beta_{42}/A\beta_{40}$ and p-tau181, suggesting a relationship between WMH volumes and neuronal injury that may be due to small vessel CVD. The reason that this relationship was not evident among participants with older ages could be that older age is associated with increased neurodegeneration due to many other processes besides CVD, thus t-tau levels become less reflective of (and less correlated with) neurodegeneration due to small vessel WMH. However, since this analysis was exploratory and the age range of participants was limited, this finding needs to be re-examined in future studies.

WMH volumes and CSF $A\beta_{42}$ and $A\beta_{42/40}$

In the current study, CSF levels of $A\beta_{42}$ and $A\beta_{42/40}$ were not associated with global or regional WMH volumes among individuals with normal cognition, consistent with several prior reports [18–21]. By comparison, other studies found that lower $A\beta_{42}$ was related to higher global WMH burden among cognitively normal older adults [13–17], and to higher regional WMH in the parietal lobe and to a smaller degree in the frontal lobe [13]. The results from the current study suggest that the reason for the discrepancy across studies is likely unrelated to participant age, vascular risk levels, presence of hypertension, or *APOE* ε4 genetic status, as we found no interactions between these variables and $A\beta_{42}$ or $A\beta_{42}/A\beta_{40}$ with respect to global or regional WMH values. However, analyses stratified by *APOE* ε4 genetic status

provided some evidence that $A\beta_{42}/A\beta_{40}$ may be more strongly associated with WMH volumes among $\varepsilon 4$ carriers than non-carriers, in line with two prior reports [17, 28]. Given that older *APOE* $\varepsilon 4$ carriers have more abnormal levels of $A\beta_{42}$ and $A\beta_{42}/A\beta_{40}$ than $\varepsilon 4$ non-carriers, this may suggest that levels of $A\beta_{42}$ have to be sufficiently abnormal (indicating higher amyloid plaque burden) before associations with WMH become evident.

It is important to note that almost two-thirds of participants in the current study were female, whereas participants in all five prior studies reporting significant association between CSF $A\beta_{42}$ and WMH among cognitively normal participants had a higher proportion of male than female participants (range = 51% to 71% male). It is possible therefore, that the relationship between $A\beta_{42}$ and WMH is stronger among males than females. Consistent with this possibility, a *post-hoc* analysis of the current data among participants with normal cognition revealed a significant association between higher global WMH volumes and lower $A\beta_{42}/A\beta_{40}$ among males ($n = 46$, *estimate* = –10.1, $SE = 4.6$, $p = 0.035$), but not females ($n = 87$, *estimate* = –3.4, $SE = 4.8$, $p = 0.49$; for $A\beta_{42}$, $p = 0.089$ for males and $p = 0.79$ for females, covarying age, education, *APOE* $\varepsilon 4$, and vascular risk score). Future studies are needed to further investigate this potential sex difference in the relationship between amyloid and WMH, as well as possible underlying mechanisms.

Our results further suggest that associations between WMH and Aβ42 or $A\beta_{42}/A\beta_{40}$ may be more evident among individuals with cognitive impairment than those with normal cognition, as lower $A\beta_{42}/A\beta_{40}$ was related to higher parietal WMH volumes only among participants with MCI. Few prior studies have directly examined whether associations of amyloid and WMH differ as a function of diagnostic status and results have been mixed. Consistent with our results, two studies found that lower $A\beta_{42}$ was associated with higher global WMH burden among participants with AD dementia, but not those with normal cognition or subjective cognitive impairment [20, 21]. By contrast, other studies observed significant relationships between lower $A\beta_{42}$ and WMH burden among both cognitively normal and MCI participants [13], among neither cognitively unimpaired or impaired participants [18], or a stronger association among those without cognitive impairment than those among impaired individuals [16, 17]. The reasons for these discrepancies across studies are unclear and but may be related to the fact that most prior studies examined global WMH levels, whereas amyloid levels may be primarily associated with WMH in the parietal lobe [7].

The mechanistic links between amyloid and WMH are not yet well understood, but may result from the accumulation of Aβ in small cerebral arteries, leading to the narrowing or occlusion of the vessels and vessel wall damage, which can cause ischemia and result in WMH, similar to the process seen in cerebral amyloid angiopathy [61]. Additionally, animal models suggest that vessel-wall damage may hinder amyloid clearance via perivascular spaces [62, 63], leading to a self-reinforcing cycle of Aβ deposition, vessel damage and consequent ischemia. It is also possible that brain amyloid accumulation directly damages white matter, leading to WMH [56]. Interestingly, *APOE* $\varepsilon 4$ carriers are not only at increased risk for AD but are also predisposed to cerebral amyloid angiopathy. This could explain the potentially stronger association between WMH and $A\beta_{42}/A\beta_{40}$ among $\varepsilon 4$ carriers than non-carriers [17, 28].

WMH volumes and vascular risk

The finding that higher vascular risk scores, and in particular hypertension, were associated with higher parietal WMH volumes (and to a lesser extent with frontal WMH volumes) among all non-demented participants and the cognitively normal subgroup, independent of AD-biomarker levels, supports the view that WMH are a marker of cerebrovascular disease. For example, many prior studies have shown that individuals with greater vascular risk, including hypertension and diabetes, have higher WMH volumes [64–66].

However, our results showed that neither levels of vascular risk, nor the presence of hypertension, modified the relationships between regional or global WMH volumes and CSF Aβ, tau, and p-tau among individuals with normal cognition. This is consistent with prior studies among non-demented participants [56, 67]). It is possible that results may differ when using quantitative measures of hypertension or other vascular risk factors, with dichotomous vascular risk indicators lacking sensitivity (e.g., [14]). Additionally, vascular risk burden was relatively low in the current sample and results may differ in populations with higher vascular risk levels.

Regional WMH: Distinct etiologies and risk factor associations

The present results support the view that WMH in different regions of the brain may have at least partially distinct etiologies and risk factor associations. For example, consistent with the present findings, cortical amyloid appears to have the greatest impact on parietal and posterior WMH [7–11]. The parietal cortex is one of the earliest regions to accumulate amyloid in AD [68] and it has been suggested that cortical Aβ may trigger axonal damage in underlying white matter due to Wallerian degeneration, giving rise to WMH [69]. Also in line with our findings, frontal WMH may be particularly associated with vascular risk factors, including hypertension, and reflective of CVD [7, 70–72], possibly because the frontal lobes are more vulnerable to arteriosclerosis due characteristics of the regional blood supply [70]. Lastly, as discussed above, the association of temporal WMH with p-tau and t-tau may reflect the fact that AD-related tau tangle pathology begins in the temporal lobe, when participants are still cognitively normal.

Discrepancies across prior studies in the relationship between WMH volumes and CSF AD biomarkers

The current study, together with results from prior studies, suggests that inconsistencies regarding associations between CSF AD biomarkers and WMH volumes may be attributed to several factors. First, associations appear to vary by participant age and disease stage, with t-tau showing stronger associations with global WMH burden among younger than older participants, and $A\beta_{42}$ and $A\beta_{42/40}$ being more strongly related to WMH in later disease stages. Second, the strength of these associations may differ across brain regions, with p-tau and t-tau primarily related to WMH in temporal lobe regions and $A\beta_{42}/A\beta_{40}$ more strongly related to parietal areas. Third, associations between CSF AD biomarkers and WMH appear to be somewhat stronger among *APOE* ε4 carriers than non-carriers, though large samples may be needed to detect this difference in the preclinical stage of disease. Fourth, there may be subtle sex differences in the relationship between amyloid and WMH burden that require further investigation. Large studies that are able to stratify analyses by these multiple factors are needed to examine these hypotheses.

Study limitations

These findings should be interpreted within the context of the study's limitations. The majority of participants were White, well-educated, and had a first-degree relative with AD-dementia. Results, therefore, may not generalize to the general population. The cross-sectional nature of the analyses precludes any inferences regarding causality. Additionally, the MCI group was small, limiting our power to detect differences by diagnostic status. Also, the study did not differentiate between periventricular and deep subcortical WMH, which have been suggested to reflect distinct etiologies and risk factor associations [7, 73]. Longitudinal studies in more diverse cohorts will be valuable in further characterizing how changes in AD biomarkers over time relate to white matter changes and WMH across the AD spectrum.

Future directions

More broadly, future studies investigating mechanistic links between AD and WMH should consider including markers of cerebral blood flow (CBF) and blood-brain barrier dysfunction. Many studies have documented decreases in CBF among individuals with AD-dementia and MCI relative to those with normal cognition, with some evidence for greater reductions among *APOE* ε4 carriers than noncarriers (reviewed in [74]). Moreover, some recent work suggests that AD-related reductions in CBF may be linked to the degeneration of pericytes [75], the cells that line the walls of blood vessels and are important for regulating cerebral blood flow and maintaining the blood-brain barrier. For example, CSF markers of pericyte degeneration have been associated with breakdown of the blood-brain barrier [76] and with cognitive decline, particularly among *APOE* ε4 carriers [77]. Other evidence from mouse models and human postmortem tissue suggests that $A\beta_{40}$ and $A\beta_{42}$ cause the constriction of pericytes, reducing their diameter and thus reducing blood flow [78]. Although the timing of events related to pericyte degeneration, blood-brain barrier breakdown, and amyloid accumulation remains unclear, breakdown of the blood-brain barriers appears to be associated with both AD biomarkers and vascular risk factors, though on different molecular scales [79]. This underscores the need for jointly investigating markers of AD and vascular function in the same study.

Summary and conclusions

In summary, our results support the view that among cognitively normal middle-aged and older individuals, WMH volumes are primarily associated with markers of neurofibrillary tau pathology (as measured by p-tau181) and neurodegeneration (as measured by t-tau), as well as with systemic vascular risk factors. Our findings suggest that once individuals develop MCI, WMH may also be associated with amyloidosis. Importantly, AD-specific pathways may contribute to the formation of WMH in a regionally-specific manner, with neurofibrillary tau pathology playing a role in temporal WMH and amyloid pathology in parietal WMH volumes.

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CONFLICT OF INTEREST

Marilyn Albert is an advisor to Eli Lilly.

All other authors have no conflict of interest to report.

DATA AVAILABILITY

Anonymized data used in the analyses presented in this report are available on request from qualified investigators (https://www.biocard-se.org).

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