

Association of Amyloid Pathology With Myelin Alteration in Preclinical Alzheimer Disease

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IMPORTANCE The accumulation of aggregated β -amyloid and tau proteins into plaques and tangles is a central feature of Alzheimer disease (AD). While plaque and tangle accumulation likely contributes to neuron and synapse loss, disease-related changes to oligodendrocytes and myelin are also suspected of playing a role in development of AD dementia. Still, to our knowledge, little is known about AD-related myelin changes, and even when present, they are often regarded as secondary to concomitant arteriosclerosis or related to aging.

OBJECTIVE To assess associations between hallmark AD pathology and novel quantitative neuroimaging markers while being sensitive to white matter myelin content.

DESIGN, SETTING, AND PARTICIPANTS Magnetic resonance imaging was performed at an academic research neuroimaging center on a cohort of 71 cognitively asymptomatic adults enriched for AD risk. Lumbar punctures were performed and assayed for cerebrospinal fluid (CSF) biomarkers of AD pathology, including β -amyloid 42, total tau protein, phosphorylated tau 181, and soluble amyloid precursor protein. We measured whole-brain longitudinal and transverse relaxation rates as well as the myelin water fraction from each of these individuals.

MAIN OUTCOMES AND MEASURES Automated brain mapping algorithms and statistical models were used to evaluate the relationships between age, CSF biomarkers of AD pathology, and quantitative magnetic resonance imaging relaxometry measures, including the longitudinal and transverse relaxation rates and the myelin water fraction.

RESULTS The mean (SD) age for the 19 male participants and 52 female participants in the study was 61.6 (6.4) years. Widespread age-related changes to myelin were observed across the brain, particularly in late myelinating brain regions such as frontal white matter and the genu of the corpus callosum. Quantitative relaxometry measures were negatively associated with levels of CSF biomarkers across brain white matter and in areas preferentially affected in AD. Furthermore, significant age-by-biomarker interactions were observed between myelin water fraction and phosphorylated tau 181/ β -amyloid 42, suggesting that phosphorylated tau 181/ β -amyloid 42 levels modulate age-related changes in myelin water fraction.

CONCLUSIONS AND RELEVANCE These findings suggest amyloid pathologies significantly influence white matter and that these abnormalities may signify an early feature of the disease process. We expect that clarifying the nature of myelin damage in preclinical AD may be informative on the disease's course and lead to new markers of efficacy for prevention and treatment trials.

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The progression of Alzheimer disease (AD) pathology occurs several years before the development of dementia. According to the amyloid-cascade hypothesis, the disruption of critical metabolic processes that lead to the ultimate neurodegeneration in AD is initiated by the accumulation of aggregated β -amyloid 1-42 ($A\beta$ 1-42) and the assembly of neurofibrillary tangles.¹ Such processes are clearly detrimental to neuronal cell bodies, dendrites, and axonal processes; however, myelin and myelin-producing oligodendrocytes may be equally vulnerable to the impairments caused by $A\beta$ and tau protein hyperphosphorylation.² White matter hyperintensities (WMHs) play a role in symptom presentation but are not core features of AD pathology.³ Postmortem and in vivo magnetic resonance imaging (MRI) studies have substantiated recent hypotheses of white matter involvement in AD, finding reduced white matter volume and alterations of white matter microstructure.⁴⁻⁶ Still, little is known about the relationship between $A\beta$ pathology and myelin alteration.

Recent advancements of cerebrospinal fluid (CSF) biomarkers show promise for early detection of AD pathology. Concentrations of specific proteins within CSF, such as $A\beta$ 42, total tau protein (Ttau), and phosphorylated tau 181 (Ptau181), are related to the core pathology of AD⁷⁻⁹ and differentiate patients with AD from healthy, age-matched controls.¹⁰ Cerebrospinal fluid biomarkers have additionally been linked to measures obtained with volumetric MRI and diffusion tensor imaging, suggesting that CSF biomarkers are sensitive to structural brain changes during the preclinical stages, onset, and progression of AD.⁴ Relationships between CSF biomarkers and brain structure are increasingly important^{4,11}; however, while MRI techniques provide detailed anatomical and microstructural insight, they are influenced by a broad range of microstructural changes.¹² Thus, the biological interpretation of such associations is challenging.

The breakdown of the myelin sheath may be an early phenomenon in AD,^{5,13} but more clinical studies are needed, especially in the preclinical stage. Recent quantitative MRI measures, such as longitudinal and transverse relaxation rates (R_1 and R_2 , respectively) and the myelin water fraction (MWF), may provide increased sensitivity to myelin content,^{14,15} and these may offer new insights regarding the role of myelin vulnerability in the pathogenesis of AD. Examining relationships between white matter measures and CSF biomarkers related to AD pathology—such as $A\beta$ 42 (reflecting cortical amyloid deposition), Ttau (as a marker for the intensity of neurodegeneration), and Ptau181 (correlating with tangle burden)¹⁶—could provide an appreciation for the extent and timing of myelinated white matter damage in AD. To our knowledge, no studies to date have explored such relations at the preclinical stage of AD. However, evidence of such relationships may support and transform the current understanding of the involvement of myelinated white matter and its alterations during the development of AD.

We report results from 71 late-middle-aged asymptomatic adults who underwent CSF collection via lumbar puncture¹⁷ and novel MRI using the Multicomponent Driven Equilibrium Single Pulse Observation of T_1 and T_2

Key Points

Question What are the underlying relationships between white matter microstructure and, more specifically, myelin content and cerebrospinal fluid biomarkers of Alzheimer disease?

Findings In this cohort study of asymptomatic individuals with genetic risk factors for Alzheimer disease, significant associations were observed between quantitative neuroimaging measures, including the myelin water fraction and cerebrospinal fluid biomarkers of Alzheimer pathology.

Meaning Mechanisms of amyloid pathology influence and alter the white matter microstructure, which may indicate an early feature of Alzheimer disease.

(mcDESPOT) technique. These data were acquired to examine AD pathology as indexed by CSF biomarkers and the MWF, a surrogate measure of myelin content.^{15,18} Guided by prior models and observations,⁴ we hypothesized that the deposition of $A\beta$ 42 and the hyperphosphorylation of tau protein would affect oligodendrocytes and alter myelin sheath integrity. Hence, we predicted decreased $A\beta$ 42 and elevated Ttau and Ptau₁₈₁ levels would be associated with decreased MWF. We additionally hypothesized that proteins that precede $A\beta$ 42 formation, including elevated cleavage of the amyloid precursor protein by β -secretase that results in higher soluble amyloid precursor protein (sAPP β), may stimulate processes of white matter alteration and be associated with myelin content alterations in the preclinical phase.

Methods

A total of 147 participants were enrolled in the study, from which 71 asymptomatic participants (19 males) between the ages of 48 and 72 years (mean [SD] age = 61.6 [6.4] years) were included based on the availability of assayed CSF and mcDESPOT imaging. Healthy community volunteers were recruited from the Wisconsin Alzheimer Disease Research Center and the Wisconsin Registry for Alzheimer's Prevention study.¹⁷ Participants underwent assessments that included neuropsychological testing, apolipoprotein E (*APOE*) genotyping, laboratory tests, clinical measurements, and comprehensive health history characterization.¹⁷ Twenty-eight participants (39%) were carriers of at least 1 *APOE* ϵ 4 allele and 54 participants (76%) had a parental history of AD. Inclusion criteria consisted of the following: a prior visit for lumbar puncture, no contraindications for MRI, and a subsequent normal MRI scan finding. Cardiovascular health was assessed using the Framingham cardiovascular risk score and further inclusion was limited to participants who scored 27 or greater on the Mini-Mental State Examination.¹⁹ Written informed consent was obtained from all study participants and the study procedures were performed under guidelines approved by the University of Wisconsin Health Sciences institutional review board. Additional participant information is available in the eMethods in the Supplement while demographic information is provided in the Table.

CSF Collection

Cerebrospinal fluid was collected via lumbar puncture using a Sprotte 25- or 24-gauge spinal needle at the L3/4 or L4/5 level of the spinal column the morning after a 12-hour overnight fast. Lumbar punctures were performed an average of 5.86 days prior to brain imaging. Approximately 22 mL of CSF were extracted, mixed, and centrifuged at 2000g for 10 minutes. Supernatants were frozen in polypropylene tubes in 0.5-mL aliquots and stored at -80°C. Samples were aliquoted in sterile polypropylene collection tubes and stored in a 280uC freezer. The samples were subsequently sent in a single batch to the Sahlgrenska University Hospital at the University of Gothenburg in Sweden, where CSF was assayed for Aβ42, Ttau, and Ptau181 using commercially available enzyme-linked immunosorbent assay methods (INNOTEST assays; Fujirebio).²⁰ Cerebrospinal fluid sAPP-β was measured using the Meso Scale Discovery Multiplex Soluble APP assay according to the manufacturer's instructions. Board-certified laboratory technicians, blinded to clinical information, analyzed all samples according to protocols approved by the Swedish Board of Accreditation and Conformity Assessment. One batch of reagents was used yielding intra-assay coefficients of less than 10% variation.

MRI Data Acquisition and Processing

Participants were imaged on a 3 T General Electric MR750 Discovery scanner (General Electric Healthcare) with an 8-channel receive-only head coil (Nova Medical). The mcDESPOT protocol included both spoiled gradient-recalled echo images and balanced steady-state free precession images acquired over multiple flip angles (eFigure 1 in the Supplement).¹⁵ All images were acquired sagittally and shared a common field of view of 25.6 cm by 25.6 cm by 16.8 cm with an isotropic voxel resolution of 2 mm³. Acquisition time for the mcDESPOT protocol was approximately 8 minutes per participant. Additional sequence-specific parameters are provided in the eMethods in the Supplement.

R₁, R₂, and MWF maps were calculated and aligned to the Montreal Neurological Institute template space, as described in eMethods in the Supplement. Total WMH volume was also measured using high-resolution T1-weighted and T2-weighted images and the Lesion Segmentation Toolbox version 1.2.2 in Statistical Parametric Mapping 8 (<http://www.fil.ion.ucl.ac.uk/spm/>), normalized by the total intracranial volume and log-transformed for the inclusion of statistical analyses.

Statistical Analysis

Relationships of Age, CSF Biomarkers, and Imaging Measures

The single greatest risk factor for AD is age,²¹ and thus our initial analyses examined the relationship between age and the CSF biomarkers as well as age and the imaging measures (MWF, R₁, and R₂). To examine the relationships between age and CSF biomarkers, Pearson correlations were calculated in R (version 3.2.1).²² Significant correlations were defined as $P < .05$, Bonferroni-corrected for 28 independent comparisons.

Table. Demographic Characteristics of Participants

Characteristic	Mean (No. of Days)
No. of participants	71
Age, mean (SD), y	61.6 (6.4)
Sex	
Male	19
Female	52
APOE4 carriers (e4e4/e4e3)/noncarriers	28 (2/26)/43
AD parental history	
Positive	54
Negative	17
Education, y	16.96 (2.57)
MMSE score	29.36 (1.00)
RAVLT score	
Trial 1	6.74 (1.90)
Trials 2-5	45.96 (6.36)
Delay	10.97 (2.88)
TMT Part B, s	60.07 (22.49)
No. of days between MRI and lumbar puncture, mean (range), d	37.22 (0-205)

Abbreviations: AD, Alzheimer disease; APOE4, apolipoprotein E4 allele; MMSE, Mini-Mental State Examination; MRI, magnetic resonance imaging; RAVLT, Rey Auditory Verbal Learning Test; TMT, Trail-Making Test.

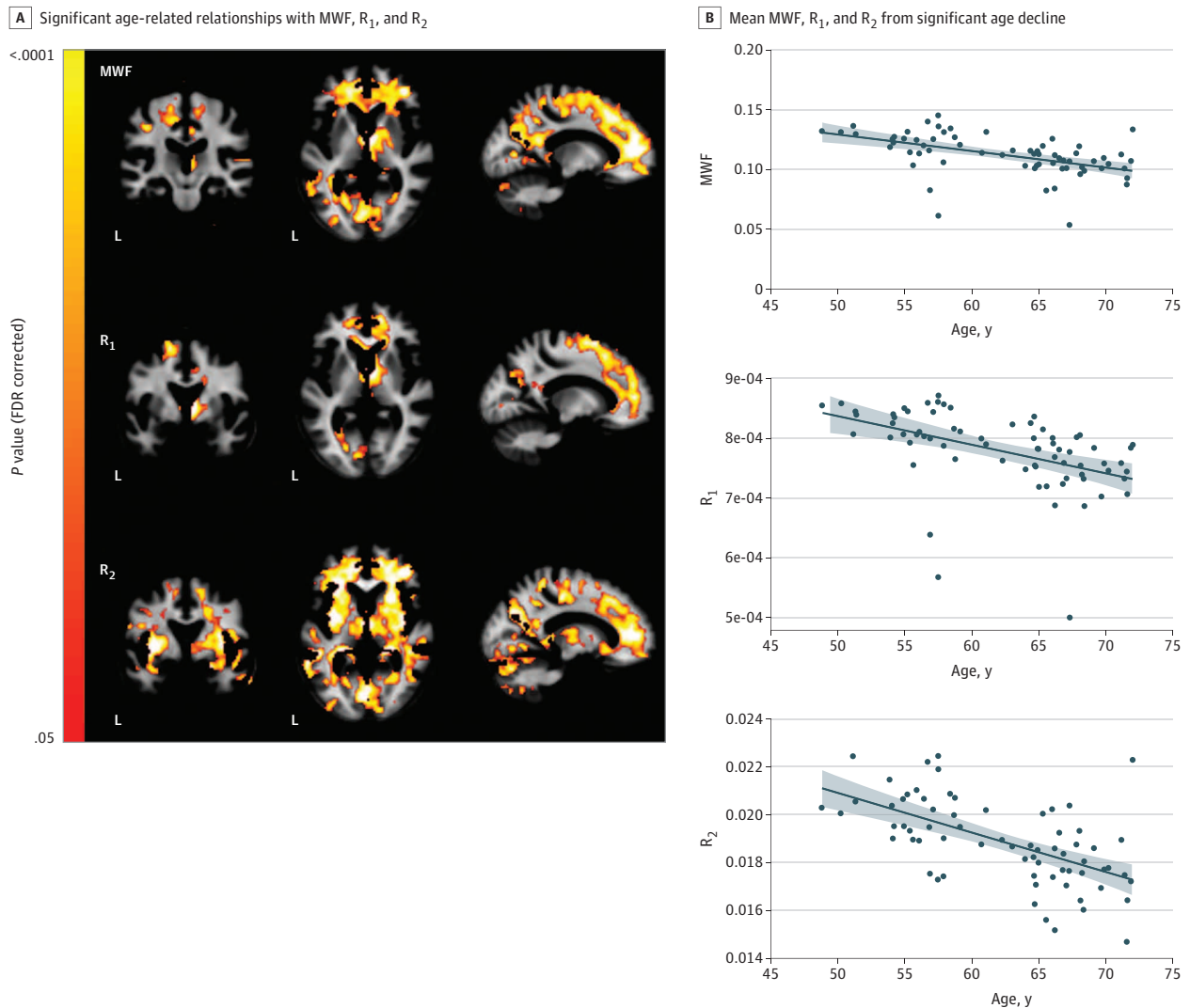
Age-related changes in R₁, R₂, and MWF were examined by fitting linear models at each brain voxel contained within a white matter mask (eFigure 2 in the Supplement). Each model examined R₁, R₂ and MWF as a function of age, while accounting for sex, APOE carrier status, log-transformed normalized WMH volume, and the Framingham cardiovascular risk score due to known effects on brain microstructure.²³ We examined education level and family history as additional nuisance variables; however, no significant relationships were observed by including these variables. Statistical maps were thresholded at a level of $P < .05$, corrected for multiple comparisons using the false discovery rate (FDR)²⁴ at the voxel level.

Relationships Between CSF Biomarkers and MWF

Voxelwise linear regressions were fit to examine relationships between R₁, R₂, and MWF and concentration levels of CSF biomarkers. Individual CSF biomarkers, as well as biomarker ratios with Aβ42, were used as predictor variables, while age, sex, APOE status, Framingham cardiovascular risk score, and log-transformed normalized WMH volume were covariates. Analyses were constrained to white matter and the FDR corrected for multiple comparisons.

After establishing significant findings between age and relaxation measures as well as between R₁, R₂, and MWF and CSF biomarkers, we used additional models to examine whether concentrations of CSF biomarkers moderated the effect of age on the brain's MWF, R₁, and R₂. Predictors in these voxelwise models included age, CSF biomarker concentration, and the interactions of CSF biomarker concentration with age, as well as the covariates of sex, APOE, log-transformed normalized WMH volume, and Framingham cardiovascular risk score.

Figure 1. Associations Between Age and Quantitative Relaxometry Measures



A, Significant ($P < .05$, false discovery rate [FDR] corrected) age-related relationships with myelin water fraction (MWF) (top row), R_1 (middle row), and R_2 (bottom row) overlaid on the group mean MWF map. Negative relationships were observed across widespread white matter, with late myelinating areas, including frontal and temporal white matter, having the strongest age-related

declines. B, representative scatterplot depicts the mean MWF, R_1 , and R_2 calculated from areas of significant age decline plotted against the age of each participant. Scatter points represent individual mean parameter (MWF, R_1 , and R_2) values and the solid trend line represents the line of best fit with age.

Results

Changes in CSF Biomarkers and Imaging Measures With Age
 Age at time of lumbar puncture was not significantly associated with CSF biomarkers (Pearson correlations shown in eTable 1 in the Supplement).

Widespread age-related changes ($P < .05$, FDR corrected) were observed, particularly in late myelinating brain regions such as frontal white matter and the genu of the corpus callosum (Figure 1). Myelin water fraction was negatively related to age, suggesting an overall decrease in myelin content with aging. R_1 decreased in similar brain regions, including frontal white matter and the genu of the corpus, while R_2 decreased

with age across most white matter. Summary information is provided in eTable 2 in the Supplement. These findings suggest alterations to microstructural white matter and perhaps specifically in myelin content; however, such changes may be a result of increases of bulk water content or other biologically based changes.²⁵ Such findings are consistent with reported literature of age-related declines of white matter during typical and atypical aging.²⁶

Relationships Between CSF Biomarkers and Imaging Measures

Voxelwise regressions between CSF biomarkers and imaging measures revealed widespread and robust main effect associations. Lower A β 42 was associated with decreased MWF, R_1 ,

and R_2 . R_2 displayed the most extensive relationships, followed by MWF and R_1 (eTables 3-5 in the Supplement). Relationships were primarily located within the left hemispheric angular gyrus white matter for MWF and R_1 , while associations with R_2 were found across the left hemispheric temporal and parietal white matter and inferior longitudinal fasciculus. Other less localized white matter regions were additionally implicated (eFigure 3 in the Supplement).

We observed significant negative relationships ($P < .05$, FDR corrected) between MWF, R_1 , and R_2 and CSF biomarkers of Ttau, Ttau/A β 42, Ptau181, Ptau181/A β 42, sAPP β , and sAPP β /A β 42. Representative findings between MWF and sAPP β /A β 42 are shown in Figure 2 while relationships between MWF, R_1 , and R_2 and other CSF biomarkers are shown in eFigures 4-6 in the Supplement. We highlight the negative association between MWF and sAPP β /A β 42 in representative scatterplots in Figure 3. Relationships with MWF were extensive across white matter and discovered in the frontal, temporal, parietal, and cerebellar white matter. Soluble amyloid precursor protein/A β 42 showed the strongest effect with regions known to be preferentially affected by AD, including temporal and frontal white matter, the body of the corpus callosum, and the cingulum.²⁷ We found negative relationships between CSF biomarkers and R_1 in the cingulum, inferior fronto-occipital fasciculus, and the superior longitudinal fasciculus, with extensive relationships occurring with sAPP β /A β 42. We also observed negative associations with R_2 , with relations between R_2 and Ptau181/A β 42 having the largest impact.

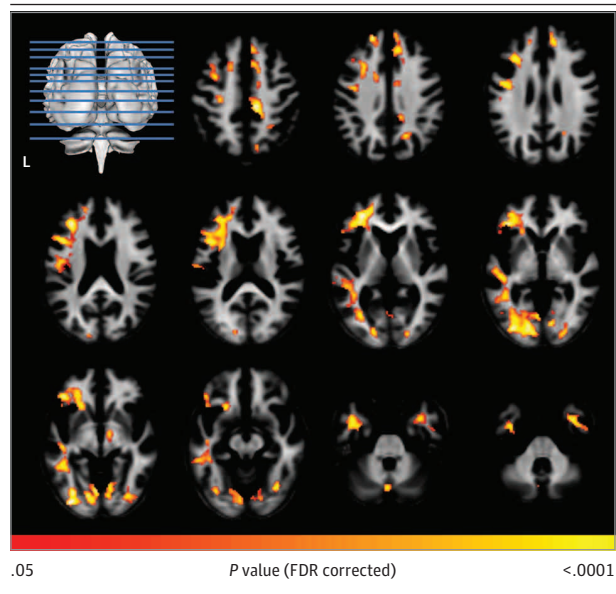
CSF Biomarkers Moderate Myelin Content Age Relationships

We observed significant ($P < .05$, FDR corrected) age-by-biomarker interactions. In particular, we found relationships between MWF and Ptau181/A β 42 in left hemispheric superior frontal gyral white matter and portions of the left superior longitudinal fasciculus in which higher levels of Ptau181/A β 42 resulted in an increased MWF decline with age (Figure 4). We provide a summary and depiction of the interaction results in eTable 6 and eFigure 7 in the Supplement.

Discussion

Alzheimer disease disrupts critical metabolic processes that ultimately lead to neurodegeneration; however, the effect of AD pathology on white matter—especially myelin—is still incompletely characterized. Using CSF biomarkers of AD together with quantitative relaxometry measures in cognitively asymptomatic middle-aged adults enriched for AD risk, we have demonstrated significant associations between AD pathology and measures of myelin health in vivo. Elevated concentrations of Ttau, Ptau181, and sAPP β —along with elevated ratios of Ttau/A β 42, Ptau181/A β 42, and sAPP β /A β 42—were robustly associated with decreased MWF, R_1 , and R_2 across widespread brain regions. The effect of age on the trajectory of MWF was moderated by ratios of Ptau181/A β 42 and Ttau/A β 42, with elevated ratios leading to accelerated MWF decline. This is the first study, to our knowledge, to demonstrate relationships between AD pathology and relaxometry measures and

Figure 2. Regional White Matter Myelin Content, as Measured by Myelin Water Fraction (MWF), Associated With Soluble Amyloid Precursor Protein/ β -Amyloid 42 (sAPP β /A β 42)

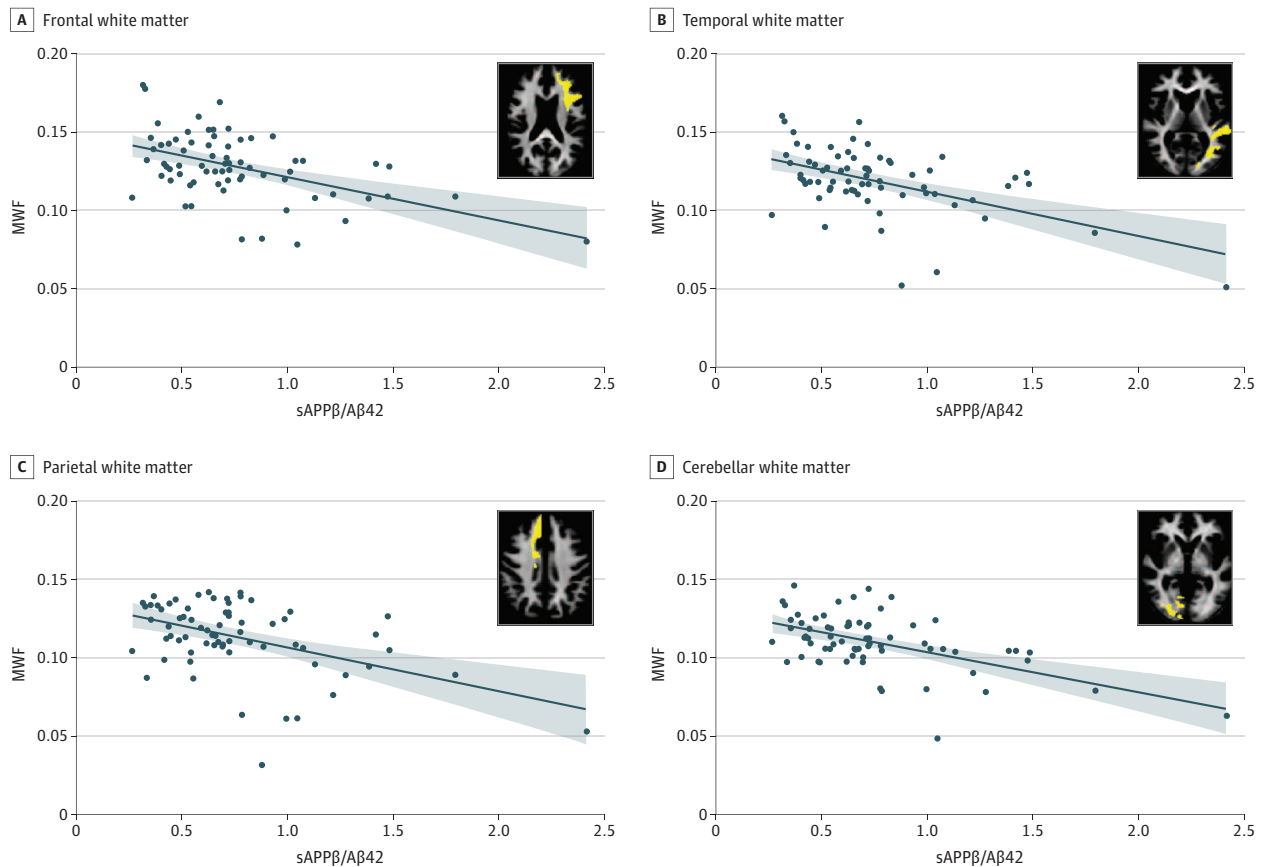


Representative axial slices depicting areas of significant relationship between MWF and sAPP β /A β 42 overlaid on the group mean MWF map. Widespread white matter was observed to be negatively related to levels of sAPP β /A β 42. Results are false discovery rate (FDR) corrected for multiple comparisons ($P < .05$).

further reinforce recent findings that AD pathology has an important impact on white matter microstructure.⁴

Our findings provide evidence that risk factors for developing AD are related to alterations of myelin content. Specifically, we find that age highly associates with MWF, R_1 , and R_2 decreases. These findings agree with studies showing white matter volume and microstructure to follow a negative gradient throughout later stages of life.^{13,28} Our results agree with hypotheses of late myelinating brain regions^{29,30} being the first to degenerate with age²⁶ and implicated in AD.³¹ Together, this suggests that white matter alterations may be centrally involved in AD pathogenesis. Analyses of MWF and age were performed using linear regressions as the age range of participants did not appear to capture the nonlinearity of the MWF trajectory. However, the brain follows a nonlinear trajectory,^{26,32} and thus future studies using larger samples and wider age ranges should examine whether MWF follows such a nonlinear trajectory. Such information would improve understanding about the timing of AD pathology, including the initial periods of myelin decline.

We additionally show robust associations between CSF biomarkers and myelin content as measured by MWF, R_1 , and R_2 . Amyloid pathology has classically been linked with processes underlying neuronal degeneration; however, studies have revealed myelin and oligodendrocytes to be especially vulnerable to impairments of A β pathology.⁵ Myelin basic protein, a canonical protein component of myelin, has recently been shown to bind A β and inhibit A β fibril formation, possibly playing a role in regulating the deposition of A β 42 and the

Figure 3. Associations Between Soluble Amyloid Precursor Protein (sAPP β)/ β -Amyloid 42 (A β 42) and Myelin Water Fraction (MWF)

Clusters of 50 voxels or greater were determined from false discovery rate-corrected associations between MWF and sAPP β /A β 42. Mean MWF from the indicated significant clusters were calculated and plotted against sAPP β /A β 42. Each point in the scatter represents mean MWF and corresponding sAPP β /A β 42 from 1 participant (n = 71).

formation of amyloid plaques in parenchyma.^{33,34} Therefore, the loss of myelin and decreases in concentrations of myelin basic protein may promote accelerated A β 42 depositions and result in increased amyloid plaque formation. Studies have additionally demonstrated that myelin abnormalities occur prior to axon defects from tau proteins inhibiting axon transport,³⁵ meaning that myelination defects precede overt amyloid and tau pathologies.³⁶

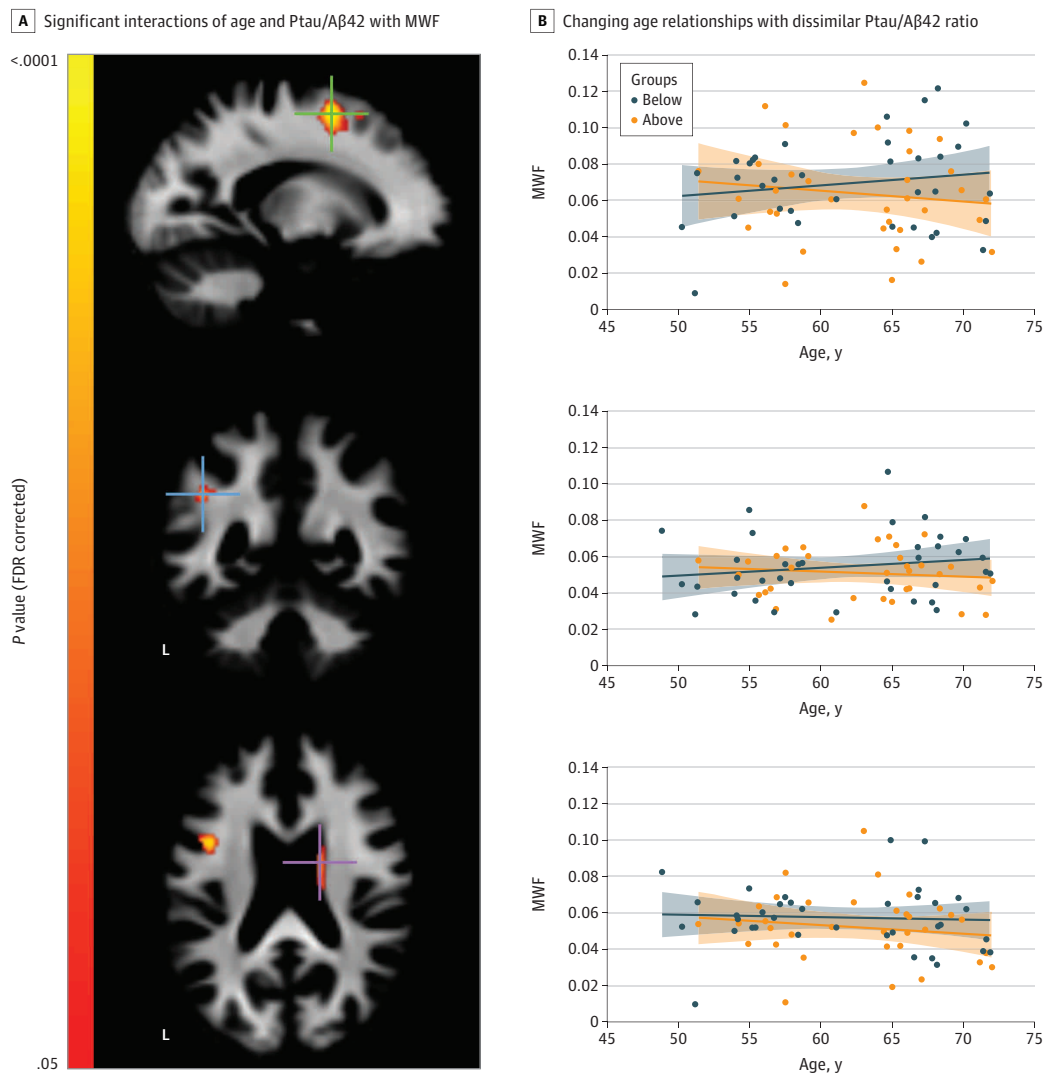
Interestingly, we observed strong associations of decreased myelin content with increased ratios of sAPP β and sAPP β /A β 42, with sAPP β /A β 42 having the greatest sensitivity. β -Amyloid is a product of amyloid precursor protein cleavage by β -secretase, which ultimately leads to the formation of amyloid plaques.⁵ Animal studies using immunohistochemistry and electron microscopy on the triple-transgenic AD mouse, which harbors the amyloid precursor protein transgene, have found localized alterations in myelination and oligodendrocyte marker expression prior to the manifestation of amyloid and tau pathologies.³⁶ Findings from these previous studies in the context of the current study raise questions about the role of sAPP β in myelin alterations of AD pathology. The remaining challenge of deciphering the causal mechanisms of

these changes is determining if aberrant proteolytic processing of the amyloid precursor protein causes subsequent myelin damage or if degradation of myelin results in altered amyloid precursor protein processing. We need further animal and human studies to elucidate this causal pathway.

Our findings also indicate that the strength of the relationship between MWF and age is altered by the ratios of Ptau181/A β 42 and Ttau/A β 42. While tau pathology may follow amyloid pathology, hyperphosphorylation of tau protein also occurs during the repair of the myelin sheath.⁵ Increased concentrations of Ttau and Ptau181 may reflect initial benefits to remyelination^{5,13} and the effects of moderate myelin breakdown with aging. One hypothesis is that extensive time-limited repair and/or unsuccessful repair could lead to protein aggregation and deposition as neurofibrillary tangles.⁵

Limitations

This study has several limitations. First, while the sample provides a powerful cohort for investigating relationships of CSF biomarkers and brain microstructure, the enriched risk for AD (large percentage of family history positive- and APOE ϵ 4-positive individuals), as well as the low percentage of males

Figure 4. Levels of Phosphorylated Tau (Ptau181)/ β -Amyloid 42 ($A\beta$ 42) Moderate Age-Related Changes of Myelin Water Fraction (MWF)

A, Representative illustration of significant interactions of age and Ptau181/A β 42 with MWF. Myelin water fraction from the peak voxel (as indicated by the cross-hair) was extracted and participants were subsequently split into 2 groups by the Ptau181/A β 42 ratio median. B, Myelin water fraction was plotted and fit against participant age for each group. These plots demonstrate the changing age relationship with dissimilar Ptau181/A β 42 ratio.

(19%), could limit the generalizability of our findings. Family history was found to have a nonsignificant effect on the findings of the current study. *APOE* status was included as a nuisance variable; however, it is possible that *APOE* allele status may impact brain aging^{26,28} and that future studies examining such relationships could be informative. Second, while qualitative agreement has been observed between mcDESPOT MWF and histology,³⁷ and strong evidence has been provided to support the application of mcDESPOT MWF,^{29,30,38-40} future studies investigating the specificity and sensitivity of mcDESPOT-derived MWF measurements compared with additional factors—such as changes to brain volume, water content, iron load, or microstructural lipids and proteins—are necessary. While there was an overlap in findings with MWF, R_1 , and R_2 , associations in different brain structures between brain measures were also observed, suggesting that these measures are complimentary

but may elucidate differential microstructural processes.²⁹ Last, cross-sectional analyses have permitted us to examine the associations between CSF biomarkers and neuroimaging measures. However, these findings should be explored in larger samples and extended in longitudinal samples to evaluate the time course of these relationships.

Conclusions

Myelin alterations in AD are suspected but understudied in human populations. Using a quantitative MRI technique sensitive to myelination, we measured CSF biomarkers and myelin content to examine the relationships between AD pathology. Our findings show, for the first time to our knowledge, that decreased concentrations of A β 42 and elevated

concentrations of Ttau, Ptau181, sAPP β , and their ratios with A β 42 are closely associated with brain myelin content. Furthermore, we show that the age-related decline in myelin content is influenced by the levels of CSF biomarkers. These

findings provide further evidence of white matter involvement, and particularly myelin content, in the pathogenesis of AD and suggest that such alterations may be one of the earliest characteristics of the disease process.

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Author Contributions: Dr Bendlin had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Acquisition, analysis, or interpretation of data: Dean, Hurley, Kecskemeti, O'Grady, Canda, Davenport-Sis, Zetterberg, Blennow, Asthana, Sager, Johnson, Alexander, Bendlin.

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