White Matter Integrity Determined With Diffusion Tensor Imaging in Older Adults Without Dementia
Influence of Amyloid Load and Neurodegeneration

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**IMPORTANCE** Pathophysiologic mechanisms leading to loss of white matter integrity and the temporal positioning of biomarkers of white matter integrity relative to the biomarkers of gray matter neurodegeneration and amyloid load in the course of Alzheimer disease (AD) are poorly understood.

**OBJECTIVE** To investigate the effects of AD-related gray matter neurodegeneration and high β-amyloid on white matter microstructure in older adults without dementia.

**DESIGN, SETTING, AND PARTICIPANTS** A population-based, longitudinal cohort study was conducted. Participants included in the Mayo Clinic Study of Aging (N = 701) who underwent magnetic resonance imaging, diffusion tensor imaging (DTI), and positron emission tomography studies with diagnoses of cognitively normal ([CN] n = 570) or mild cognitive impairment ([MCI] n = 131) were included. Both groups were divided into biomarker-negative, amyloid-positive-only, neurodegeneration-positive-only, and amyloid plus neurodegeneration-positive groups based on their amyloid load shown on carbon 11-labeled Pittsburgh Compound B positron emission tomography, AD hypometabolic pattern shown on fludeoxyglucose F 18 positron emission tomography, and/or hippocampal atrophy shown on magnetic resonance imaging.

**MAIN OUTCOMES AND MEASURES** Fractional anisotropy (FA) determined using DTI.

**RESULTS** No FA alterations were observed in biomarker-negative MCI and amyloid-positive-only CN and MCI groups compared with biomarker-negative CN participants on voxel-based analysis (P < .05; familywise error corrected). Conversely, the neurodegeneration-positive-only and amyloid plus neurodegeneration-positive CN and MCI groups consistently had decreased FA in the fornix, which correlated with cognitive performance (ρ = 0.38; P < .001). Patients with MCI had more extensive white matter involvement than did those with CN, and the greatest FA decreases were observed in the amyloid plus neurodegeneration-positive MCI group (P < .05; familywise error corrected).

**CONCLUSIONS AND RELEVANCE** A high amyloid load does not influence diffusion tensor imaging-based measures of white matter integrity in the absence of coexistent gray matter neurodegeneration in older adults without dementia.
Diffusion tensor imaging (DTI) shows the profound loss of white matter integrity in Alzheimer disease (AD) starting from the prodromal stages. A decrease in the directionality of water diffusion measured with fractional anisotropy (FA) on DTI has been linked to a loss of myelin and axons in the white matter. According to the biomarker model of AD and the preclinical staging of AD derived from this model, alterations in biomarkers of gray matter neurodegeneration follow biomarkers of amyloid deposition during the course of disease progression from preclinical to clinical dementia. However, the AD-related pathophysiologic mechanisms leading to loss of white matter integrity and the temporal positioning of biomarkers of white matter integrity relative to the biomarkers of gray matter neurodegeneration and amyloid load in the course of AD are unknown.

In this study, we investigated the FA alterations in cognitively normal (CN) older adults and elderly individuals with mild cognitive impairment (MCI) from a population-based study on older adults without dementia who were classified as neurodegeneration positive or neurodegeneration negative and/or who were amyloid positive or amyloid negative. Our objective was to determine the effects of AD-related gray matter neurodegeneration and high β-amyloid levels on white matter microstructure in older adults without dementia.

Methods

Participants

Older adults (701 individuals aged 70-89 years) who participated in the Mayo Clinic Study of Aging (MCSA) magnetic resonance imaging (MRI) and positron emission tomography (PET) studies from November 1, 2009, to August 31, 2013 were included. The MCSA is a prospective, population-based study of older adults without dementia in Olmsted County, Minnesota. All study procedures and ethical aspects of the MCSA were approved by the institutional review boards of the Mayo Clinic and Olmsted Medical Center. All persons examined as part of the study were informed of the scope of the project and signed an informed consent form. To be included in the present study, participants in the MCSA should have been part of MRI, amyloid PET with carbon 11-labeled Pittsburgh Compound B ([11C]-PiB), and fludeoxyglucose F 18 (FDG) PET studies during the same cycle of clinical evaluation. The neuropsychological test scores were scaled such that they had a mean (SD) of 0.0 (1.0) among all MCSA full participants with a mean determined to obtain a global cognitive z score. The diagnosis of MCI was based on the published criteria: cognitive complaint, cognitive function not normal for age, decline in cognition, essentially normal functional activities, and not demented. The diagnosis of dementia was based on the Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition) criteria, and patients with dementia were excluded. Diagnosis was determined by a consensus committee including the neurologist, neuropsychologist, and nurse who evaluated each participant, shielded from prior diagnosis. Individuals who had a contraindication for MRI, such as a pacemaker, or who were unable to participate in imaging studies because of severe illness were excluded. Patients with neurologic, psychiatric, or systemic illnesses were not excluded to preserve the representativeness of the study sample as much as possible.

Biomarker Group Classifications

Magnetic resonance imaging was performed using 3-T scanners (Signa; GE Healthcare) equipped with an 8-channel phased array coil (GE Healthcare). A 3-dimensional high-resolution magnetization-prepared rapid gradient echo acquisition was performed for hippocampal volume measurements and for anatomatic segmentation and labeling of the DTI and PET scans. Hippocampal volume was measured with FreeSurfer software, version 5.3. We calculated an adjusted hippocampal volume as the residual from a linear regression of hippocampal volume vs total intracranial volume.

Positron emission tomographic images were acquired using a PET/computed tomography scanner (DRX; GE Healthcare) operating in 3-dimensional mode. After a 40-minute uptake period, a 20-minute PiB scan was obtained. The amyloid PET acquisition consisted of four 5-minute dynamic frames, acquired from 40 to 60 minutes after injection. The FDG PET images were obtained 1 hour after the PiB scan. Quantitative analysis was performed using the fully automated image processing pipeline of PiB PET that has been described in detail. Briefly, a cortical global amyloid PET standardized uptake value ratio was obtained by combining the prefrontal, orbitofrontal, parietal, temporal, anterior cingulate, and posterior cingulate/precuneus region of interest (ROI) values normalized by the cerebellar gray matter ROI of an atlas modified in-house. The FDG PET scans were analyzed in a similar manner using angular gyrus, posterior cingulate, and inferior temporal cortical ROIs to define an AD signature composite by Landau et al normalized to the pons and vermis.

Hippocampal atrophy noted on MRI and/or hypometabolism in the AD signature composite on FDG PET was used to classify participants into the neurodegeneration-positive group, and high amyloid load noted on PET was used to classify participants into the amyloid-positive group. Cut points for amyloid positivity, hippocampal atrophy, and AD signature hypometabolism were determined from the 10th percentile of the measurement distributions in patients with clinically diagnosed AD as previously described.

DTI Methods

Acquisition of DTI was a single-shot echo-planar pulse sequence performed in the axial plane using parallel imaging with a sensitivity encoding factor of 2; repetition time, 10 200 milliseconds; in-plane matrix, 128/128; and field of view, 35 cm. The DTI volumes consisted of 41 diffusion-encoding gradient directions and 5 volumes of nondiffusion T2-weighted images. The section thickness was 2.7 mm, corresponding to 2.7-mm isotropic resolution. We used a previously tested and validated method to process DTI scans. Briefly, DTI images were corrected for patient motion and residual eddy current distortion by affine registering each volume to the first image volume, which had no diffusion weighting. Diffusion tensors were fit for extracted voxels using linear least-squares optimization, and FA images were calculated from the...
eigenvalues of the tensors using FSL, version 4 (FMRIB Software Library; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/). The Advanced Normalization Tools–Symmetric Normalization, version 1.9.29 algorithm was used for generating a study-specific template from all FA images and for nonlinear registration of a participant’s FA images to the template, and the algorithm was smoothed with an 8-mm full-width at half-maximum gaussian kernel. All CN and MCI biomarker groups were compared with the biomarker-negative CN group adjusting for age as set at $P < .05$ corrected for multiple comparisons using familywise error. The mean of the FA values derived from right and left hemispheric white matter JHU atlas ROIs was determined, and the areas under the receiver operating characteristic curve (AUROC) for distinguishing the CN and MCI biomarker groups from the biomarker-negative CN group were calculated for each ROI. The JHU atlas-based FA values were compared among the CN and MCI biomarker groups with the biomarker-negative CN control group and ranked according to AUROC values. Regions with an AUROC greater than 0.70 were displayed on a rendered single-participant transparent brain for visualization.

**Results**

**Participant Characteristics**

Participant characteristics classified according to the biomarker positivity are listed in Table 1 for the CN group and Table 2 for the MCI group. Of 570 CN participants, 258 (45.3%) were classified as biomarker negative and 77 (13.5%) were classified as amyloid and neurodegeneration positive. In contrast, a smaller proportion of MCI participants (21 of 131 [16.0%]) were classified as biomarker negative and a larger proportion of MCI participants were classified as amyloid and neurodegeneration positive (55 of 131 [42.0%]). Biomarker-negative participants were younger than the biomarker-positive participants in both the CN ($P < .001$) and MCI ($P = .002$) groups. Because of age differences among the biomarker groups, we used age as a covariate in all DTI analysis. The APOE ε4 allele was more frequent in the amyloid-positive compared with the amyloid-negative CN and MCI groups ($P < .001$). Cognitive performance measured with the global cognitive z score declined from biomarker-negative to only amyloid-positive to only neurodegeneration-positive to neurodegeneration plus amyloid-positive groups in the CN ($P < .001$) and MCI ($P = .03$) participants. Similar frequencies of amnestic and nonamnes-
tic MCI subtypes were observed among the 4 biomarker MCI groups (P = .91).

**Voxel-Based Analysis**

Voxel-based analysis did not reveal any differences in FA values when the amyloid-positive-only CN group was compared with the biomarker-negative CN control group adjusted for age (P > .05; familywise error corrected). However, lower FA was observed in both of the CN neurodegeneration-positive groups. The CN participants classified as neurodegeneration-positive only had decreased FA in the fornix and focal areas in the corpus callosum and occipital white matter compared with the biomarker-negative CN controls. The CN participants classified as neurodegeneration-positive plus amyloid positive had similarly decreased FA in the fornix and slightly greater involvement in the corpus callosum and occipital lobe white matter. Involvement of the right parahippocampal white matter was also observed in CN individuals classified as neurodegeneration plus amyloid positive (Figure 1A).

Voxel-based analysis findings adjusted for age in participants with MCI showed similarities to the CN group. There were no differences in FA values when the biomarker-negative and the amyloid-positive-only MCI group was compared with the biomarker-negative CN control group (P > .05; familywise error corrected). However, MCI individuals classified as neurodegeneration-positive had decreased FA in the fornix, corpus callosum, focal areas in the cingulate gyrus, and occipital lobe white matter compared with the biomarker-negative CN controls. The MCI participants classified as neurodegeneration plus amyloid positive had similarly decreased FA in the fornix, corpus callosum, cingulate gyrus, and occipital lobe white matter, but they also had decreased FA in the precuneus, basal frontal, and temporal lobe white matter (Figure 1B).

**Atlas-Based Analysis**

To determine the specific white matter tracts that were involved in the neurodegeneration-positive CN and MCI biomarker groups, we performed a secondary JHU atlas-based analysis on participant FA maps and reported the individual regional white matter FA values that distinguished the neurodegeneration-positive (amyloid-positive or -negative) CN and MCI groups from the biomarker-negative CN group with an AUROC of greater than 0.70. Right and left hemispheric tract FA values, 3 sections of the corpus callosum ( genu, body, and splenium), and the mean of the 2 sections of the cingulum tract (hippocampal and cingulate gyrus) in the JHU atlas was determined.

The only tract that distinguished CN participants in the neurodegeneration-positive groups from those in the biomarker-negative group was fornix (AUROC, 0.79 for amyloid-positive; AUROC, 0.74 for amyloid-negative). Similarly, fornix was the only tract that distinguished the neurodegeneration-positive MCI group from the biomarker-negative CN group with the highest AUROC (0.87 for amyloid-positive vs 0.83 for amyloid-negative). There were additional tracts and white matter regions that distinguished the neurodegeneration-positive MCI group from the biomarker-negative CN group with an AUROC greater than 0.70 that are displayed on a single-participant-rendered brain in Figure 2. Similar to our findings in voxel-based analysis, there were more extensive FA decreases in the white matter in neurodegeneration-positive and amyloid-positive MCI participants compared with those who were only neurodegeneration-positive.

**Fornix FA and Correlations With Cognitive Function**

Fornix was the only tract that consistently showed lower FA values in the neurodegeneration-positive CN and MCI groups compared with the biomarker-negative CN group regardless of...
of amyloid biomarker status. Because positivity for gray matter neurodegeneration was determined based on the presence of hippocampal atrophy noted on MRI and/or hypometabolism in the AD signature composite on FDG PET, we further...
investigated the gray matter neurodegeneration biomarkers that were associated with decreased fornix FA. In that investigation, we found that individuals with hippocampal atrophy only and those with hypometabolism in the AD signature composite only had decreased FA in the CN and MCI groups compared with biomarker-negative CN controls \((P < .001)\). In participants who had both hippocampal atrophy and hypometabolism in the AD signature composite, the FA was even lower compared with the FA in biomarker-negative CN controls \((P < .001)\) (Figure 3).

Lower FA values were associated with lower cognitive performance in the whole group with Spearman rank correlation \((\rho = 0.38; 95\% \text{ CI, 0.31 to 0.45}; P \leq .01)\) and in CN participants \((\rho = 0.30; 95\% \text{ CI, 0.22 to 0.38}; P \leq .01)\). This correlation between lower FA values and lower cognitive performance was weaker in individuals with MCI \((\rho = 0.15; 95\% \text{ CI, } -0.04 \text{ to } 0.33); P = .11\) (eFigure in the Supplement).

### Discussion

In a cohort of older adults without dementia from the community, classified according to the status of neurodegeneration and amyloid biomarkers, loss of white matter microstructural integrity noted on DTI was associated with biomarkers of gray matter neurodegeneration but not with amyloid biomarker positivity. As expected, MCI participants had more extensive white matter involvement compared with CN individuals. We found consistent decreases in fornix FA in both the CN and MCI neurodegeneration-positive groups, which correlated with cognitive performance in the entire cohort.

A high amyloid load alone did not have an effect on the microstructural integrity of the white matter in the absence of gray matter neurodegeneration in both the CN and MCI participants. Evidence from prospective cohort studies\(^{29-32}\) indicates that brain amyloidosis is not a benign process. Those with a high amyloid load are at an increased risk for cognitive decline, MCI, or dementia. However, the effects of β-amyloid on white matter appear to be associated with gray matter neurodegeneration. We found more-extensive white matter FA decreases in neurodegeneration-positive MCI participants compared with neurodegeneration-positive CN individuals. In addition, MCI participants with gray matter neurodegeneration and amyloid biomarker positivity had more widespread white matter FA decreases than did MCI participants with gray matter neurodegeneration alone. The interaction between biomarkers of neurodegeneration and β-amyloid load and their association with adverse cognitive outcomes in the course of AD have been observed and discussed\(^{28-31}\) and are consistent with our observations. There was more white matter involvement with a high β-amyloid load only in individuals with neurodegeneration-positive MCI compared with the biomarker-negative CN group. However, in the absence of cognitive impairment, a high β-amyloid load did not appear to have any additional effect on white matter integrity. In preclinical AD, the integrity of white matter is associated with gray matter neurodegeneration rather than β-amyloid; however, β-amyloid appears to be related to white matter integrity as the disease progresses and individuals develop cognitive impairment.

Fornix FA was consistently decreased in the neurodegeneration-positive CN and MCI groups compared with the biomarker-negative CN group. Decreased fornix FA is one of the earliest MRI abnormalities observed in individuals with normal cognition who are at an increased risk for AD. Decreases in fornix FA have been observed in presymptomatic carriers of familial AD mutations\(^{32}\) and in patients with MCI,\(^{5,33,34}\) which predicted the decline in memory function.\(^{5}\) Fornix carries the efferent projections from the CA1 and CA3 pyramidal neurons of the hippocampus and subiculum, connecting these structures to the septal nuclei, anterior thalamic nucleus, mammillary bodies, and medial hypothalamus. Fornix also carries the afferent cholinergic and γ-aminobutyric acid-ergic projections from the medial septal nuclei and the adjacent diagonal band back to the medial temporal lobe, interconnecting the core limbic structures.\(^{35}\) Fractional anisotropy measurements from the body of fornix are further protected from the noise of crossing fibers, which makes fornix an ideal anatomic structure for assessing microstructural changes with DTI. Because fornix carries the axons projecting from the CA1 and
CA3 pyramidal neurons in the hippocampus, the integrity of the fornix is in part linked to the integrity of the hippocampus. In keeping with that, we found significantly reduced fornix FA in individuals with hippocampal atrophy. However, in addition, fornix FA was decreased in those with reduced metabolism in the AD signature composite regions on FDG PET, even in the absence of hippocampal atrophy. The association of DTI-based white matter integrity biomarkers with gray matter neurodegeneration biomarkers is consistent with previous reports on the association of decreased white matter FA with gray matter hypometabolism and atrophy.

We found correlations between fornix FA and lower cognitive performance in the entire cohort and in the CN participants, but this relationship was weaker in those with MCI. The etiology underlying MCI is heterogeneous. Although the most common etiology underlying MCI is AD, vascular and Lewy body disease pathologies in addition to AD are common. These additional pathologies may have a significant effect on cognitive performance in MCI independent of AD-related neurodegeneration. Thus, the weaker relationship between fornix FA and cognitive performance that we observed in MCI compared with CN individuals may be related to a greater pathologic heterogeneity in MCI that affects cognitive performance. Similarly, white matter integrity may be affected by other pathologies commonly found in older adults, such as Lewy body disease, cerebrovascular disease, hippocampal sclerosis, transaxial response DNA binding protein 43 (TDP 43), and argyrophilic grain disease along with AD-related pathology. The interaction of these other pathologies with AD-related pathology and their influence on white matter integrity requires further investigation.

We did not analyze the data on mean diffusivity, which is significantly affected by partial volume averaging of the cerebrospinal fluid, especially in the fornix that is surrounded by cerebrospinal fluid. Although the influence of partial volume averaging of cerebrospinal fluid is less on FA, up to 16% of the difference in fornix FA among MCI and CN individuals has been attributed to macrostructural changes in the fornix and associated partial volume averaging of cerebrospinal fluid. Therefore, a small percentage of the differences in fornix FA that we observed among the biomarker groups may be attributed to macistrucural changes. Although we used an optimized DTI sequence for clinical applications, higher angular and spatial resolution that allows effective partial volume correction within clinically applicable time frames may improve the sensitivity of DTI to alterations in white matter microstructure in preclinical AD and MCI.

Conclusions

Data from the present study indicate that loss of white matter integrity measured with DTI accompanies gray matter neurodegeneration biomarker abnormalities and not amyloid biomarker positivity in the course of preclinical AD. Putting our findings into context with the preclinical staging of AD, loss of white matter integrity on DTI should be observed at stage 2 along with gray matter degeneration and amyloid biomarker positivity. However, 21% of the CN participants did not fit into the preclinical stages of AD because they were classified as neurodegeneration positive but amyloid negative and had FA reductions mostly confined to the fornix. Whether these CN individuals have non-AD-related pathology, which we labeled as suspected non-AD pathology, or a neurodegeneration-first pathway to AD requires further investigation with pathologic confirmation.

ARTICLE INFORMATION

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Study concept and design: Kantarci.
Acquisition, analysis, or interpretation of data: All authors.
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REFERENCES

1. Fellgiebel A, Dellani PR, Grevers D, Scheurich A, Stoeter P, Müller MJ. Predicting conversion to...
dementia in mild cognitive impairment by volumetric and diffusivity measurements of the hippocampus. Psychiatry Res. 2006;146(3):283-287.


