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Longitudinal, region-specific course of diffusion tensor imaging measures in mild cognitive impairment and Alzheimer's disease

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Abstract

Background—Diffusion tensor imaging (DTI) is a promising method for identifying significant cross-sectional differences of white-matter tracts in normal controls (NC) and those with mild cognitive impairment (MCI) or Alzheimer's disease (AD). There have not been many studies establishing its longitudinal utility.

Methods—Seventy-five participants (25 NC, 25 amnesic MCI, and 25 AD) had 3-Tesla MRI scans and clinical evaluations at baseline and 3, 6, and 12 months. Fractional anisotropy (FA) and mean diffusivity (MD) were analyzed at each time-point and longitudinally in eight *a priori*-selected areas taken from four regions of interest (ROIs).

Results—Cross-sectionally, MD values were higher, and FA values lower in the fornix and splenium of the AD group compared with either MCI or NC ($P < .01$). Within-group change was more evident in MD than in FA over 12 months: MD increased in the inferior, anterior cingulum, and fornix in both the MCI and AD groups ($P < .01$).

Conclusions—There were stable, cross-sectional, region-specific differences between the NC and AD groups in both FA and MD at each time-point over 12 months. Longitudinally, MD was a better indicator of change than FA. Significant increases of fornix MD in the MCI group suggest this is an early indicator of progression.

Keywords

Longitudinal; Alzheimer's; disease; DTI; Anisotropy; Diffusivity

1. Introduction

Alzheimer's disease (AD), a progressive neurodegenerative disease, currently affects an estimated 5.4 million Americans and this number is expected to double every 20 years [1]. Patients with mild cognitive impairment (MCI) are at a higher risk for progressing to AD and, as such, MCI is considered to be a prodromal stage [2–5]. The standard of assessment and diagnosis of both MCI and AD remains based on clinical grounds. The recently revised

National Institute on Aging and Alzheimer's Association criteria for the diagnosis of MCI and AD, in research, place new emphasis on biomarkers of AD pathophysiology [6–10]. Although the use of biomarkers for an MCI or AD diagnosis may be considered a major step forward, there is a need for continued development of accurate and reliable technologies with longitudinal utility for prognosis or use in disease-modifying treatment trials.

Diffusion tensor imaging (DTI) is a method of measuring white-matter (WM) integrity when conventional magnetic resonance imaging (MRI) has insufficient contrast to delineate WM fiber tract organization [11–13]. Fractional anisotropy (FA) and mean diffusivity (MD) are two DTI measures used to quantify the integrity of WM microstructure by measuring the relative random motion of water in cerebral tissue. These measures are reported in scalar values. Higher values of FA and lower values of MD are thought to represent normal tissue cytoarchitecture where the random motion of water along a healthy axon, for example, is tightly constrained and restricted to movement in one direction (anisotropic). FA and MD measures do not necessarily correlate, but are complementary in determining WM tract integrity. In select brain regions, both measures have been shown to differ between cognitively normal controls (NC) and persons with AD and MCI. One study reported lower FA in MCI and AD groups compared with NC in areas posterior to the anterior commissure and corresponding to corticothalamic and thalamocortical connections through the internal capsule [14]. Others have found lower FA and higher MD in subjects with AD compared with NC in the cingulum bundle [15–18] frontotemporal areas, cortical regions [16, 17, 19–21], and the splenium of the corpus callosum [19, 22–25]. Fewer studies have investigated WM changes in MCI [5, 26]. Compared with NCs, MCI patients had significant FA and MD differences in multiple cortical regions [16, 21], the splenium of the corpus callosum [21, 27], and the cingulum bundle [28].

In an earlier study we examined cross-sectional changes in the WM of eight *a priori*-selected regions of interest (ROIs) at baseline and 3 months later [29]. There were significant cross-sectional differences in fiber tract integrity (reduced FA) in the fornix, anterior cingulum, and splenium when comparing NCs to MCI or AD patients. Over a 3-month follow-up interval, during which participants remained clinically stable, FA in the anterior cingulum changed more in MCI compared with NCs, but not in AD patients. Although these results were informative, longer DTI follow-up studies are needed to fully characterize longitudinal WM changes and to determine the stability of the cross-sectional findings over time. To date, there has been only one longitudinal study of WM structure in NC compared with MCI patients [30]. FA reductions, predominantly in the corpus callosum, were reported over 13–16 months, during which time both groups remained clinically stable. MD was not examined in that study.

The present study builds on these findings by examining longitudinal DTI changes in NC, MCI, and AD using both FA and MD as indicators of WM integrity. We hypothesized *a priori* that the significant cross-sectional FA and MD differences between diagnostic groups observed at 0 and 3 months would remain at 6 and 12 months in the same regions. Further, we expected that longitudinal changes in FA and MD over 12 months would be most apparent in the MCI and AD groups in the fornix, anterior cingulum, and splenium.

2. Methods

2.1. Subjects

Participants were community-dwelling volunteers who enrolled in a longitudinal study examining the utility of neuroimaging measures as biomarkers of AD progression. Study methods have been detailed elsewhere [29]. Briefly, participants were recruited through the clinical core of the Johns Hopkins Alzheimer's Disease Research Center and evaluated

comprehensively (including an extensive neuropsychologic battery). Control (NC) participants were cognitively normal and had a Clinical Dementia Rating (CDR) [31, 32] of 0. Participants with *amnestic* mild cognitive impairment (MCI) did not have dementia, had mild memory problems, a CDR 5 0.5, and met Mayo criteria for amnestic MCI, in single or multiple domains [26]. Mild AD participants had a CDR 5 1 and met NINCDSADRDA criteria for probable AD [33]. All participants were >55 years of age, had no history of a neuropsychiatric disease other than AD, and had an informant who could provide information about their daily function. Informed consent was obtained prior to initiation of the study in accordance with the institutional review board requirements of Johns Hopkins. Consent procedures followed the guidelines endorsed by the Alzheimer's Association for participation of cognitively impaired individuals [34].

2.2. Study design

Each participant received a detailed medical evaluation at baseline, and then 3, 6, or 12 months later. Each evaluation included the following: (1) medical, psychiatric, and neurologic history; (2) comprehensive neuropsychologic battery; (3) physical, psychiatric, and neurologic examination; (4) assessment of clinical severity using the CDR scale; (5) MRI scan; and (6) blood draw. Based this clinical information, diagnoses of NC, MCI, or AD were issued at each visit.

2.3. DTI image acquisition

MRI images were acquired on a 3.0-Tesla (3T) scanner (Philips Medical Systems, Best, The Netherlands) at the F.M. Kirby Research Center for Functional Brain Imaging at the Kennedy Krieger Institute. At each scanning session, both magnetization prepared rapid gradient recalled echo (MPRAGE) and DTI scans were obtained. DTI images were acquired using a SENSE head coil on a 3T Philips MRI scanner equipped with dual-quasar gradients (up to 80 mT/m). A single-shot spin-echo–echoplanar sequence (SE-EPI) was used with diffusion gradients applied in 32 noncollinear directions and where $b = 700 \text{ s/mm}^2$. Five additional reference images with least diffusion weighting ($b = 33 \text{ s/mm}^2$) were also obtained. Fifty-sixty axial slices were acquired to cover all cerebral hemispheres and the cerebellum, parallel to the AC–PC line. The field of view, size of acquisition matrix, and slice thickness were $212 \times 212 \text{ mm}$, 96×96 , and 2.2 mm, respectively. Other imaging parameters included TR > 7000 ms, TE = 80 ms, and SENSE reduction factor = 2.5. To improve the signal-to-noise ratio, two datasets were acquired, leading to a total acquisition time of 7 minutes. It should be noted that the “real” brain orientation inside the scanner does not affect the oblique slice, because the gradient table is dynamically rotated on the oblique slice angles in such a manner that the “X gradient” is always X (right–left) of the image.

2.4. DTI data processing

The DTI datasets were transferred to a personal computer running a Windows platform and processed using DtiStudio (mri.kennedykrieger.org or www.DtiStudio.org) [35]. Images were first realigned by affine transformation, using automatic image registration [36] to remove any potential small bulk motion and Eddy-current distortion. The six elements of the diffusion tensor were calculated for each pixel using multivariate linear fitting. After diagonalization, three eigenvalues and eigenvectors were obtained. For the anisotropy map, fractional anisotropy (FA) [19] was used [37]. The eigenvector (v_1) associated with the largest eigenvalue was used as an indicator for fiber orientation. A 24-bit color-coded orientation map was created by assigning red, green, and blue channels to the x (right–left), y (anterior–posterior), and z (superior–inferior) components of the v_1 and its intensity was modulated by FA.

2.5. Regions of interest

Protocols were developed to identify specific fiber tracts and to manually delineate regions of interest within fiber tracts using the in-house software MriStudio/RoiEditor (www.MriStudio.org). ROI determination was guided by FA images generated by the software as well as DTI color maps. ROIs were drawn manually using a standardized protocol based on location, color, and size. As previously described [29], two independent operators manually drew eight ROIs with high reliability (mean interclass correlation = 0.87, range 0.82–0.95). For follow-up scans after baseline, slices were chosen by how closely they corresponded to the most recent visit.

The eight ROIs were from four fiber tracts and identified as follows [29]: (1) *fornix*—the body of the fornix drawn in two adjacent axial slices (FX1 and FX2) using the ventral midbrain and splenium of the corpus callosum as anatomic landmarks; (2) *cingulum bundle*—inferior (CG1) at the same axial slice as the cerebral peduncles, the posterior portion of the cingulum bundle (CG2 and CG3) drawn on the same two adjacent axial slices as the fornix, and the anterior portion of the cingulum bundle (CG4) identified in a coronal slice at the level of the anterior commissure; (3) *splenium* (SP)—midsagittal slice of the splenium identified as the enlarged, caudal-most region of the corpus callosum; and (4) *cerebral peduncles* (CP)—the cerebral peduncles, identified on an axial slice just inferior to the decussation of the superior cerebellar peduncles, serving as the control region.

With the exception of the splenium and the fornix, all ROIs were drawn on both the right and left hemispheres and the two sides were averaged in the analyses presented.

2.6. Longitudinal analysis

Differences in baseline characteristics between NC, MCI, and AD groups were examined using Fisher's exact tests for categorical variables and analyses of variance (ANOVAs) for continuous variables, with t tests for pairwise comparisons when a significant ($P < .05$) difference was noted. The method of generalized estimating equations (GEEs) [38] was used to address correlation between measures on the same individual across time-points. The correlation was modeled using an exchangeable correlation matrix. Using GEE, the calculated value, δ , represents fitted mean changes over time as a function of the β statistic, but not β itself. These were calculated as a linear combination of the fitted mean change over time for NC and, where applicable, the estimated difference in mean change over time for either MCI or AD. Longitudinal GEE data techniques were used to handle correlation for multiple observations within individuals. In these analyses, an exchangeable working correlation structure was used to handle correlated measurements within subjects. Robust variance estimates were included because they assure that the inferences are valid even when the assumptions on the working correlation structure are misspecified. All the techniques used in the GEE analysis were in a semiparametric model and did not require normality assumptions in measurements. For group comparisons (NC, MCI, and AD) of the DTI, clinical, and cognitive measures, Wald tests of regression coefficients were used. Covariates for analyses incorporating the DTI measures included baseline age and the number of voxels in the ROI. FA and MD measurements are reported as averages between left and right hemispheres, after being analyzed separately and finding little difference between the two. All computations were done using STATA, version 11.0 (StataCorp, College Station, TX).

3. Results

3.1. Subject characteristics

A total of 75 participants were recruited (25 NC, 25 MCI, and 25 AD) (Table 1). Baseline and 3-month characteristics have been reported previously [29]; we present baseline to 12-month characteristics here by baseline diagnosis. Briefly, demographic characteristics, including age, gender, race, and education, did not differ between groups. The occurrence of vascular conditions such as hypertension also did not differ between the groups. However, as expected, AD patients were more cognitively impaired than MCI and NC subjects (see Table 1).

At baseline, DTI data from 2 AD participants could not be processed. By 3 months, 1 MCI withdrew, 1 AD withdrew, and 1 additional AD participant had DTI data that could not be processed. By 6 months, 1 AD and 1 MCI participant withdrew. There were no additional dropouts at 12 months. Those with missing DTI data at baseline were not the same as those with missing data at 3 or 6 months. The longitudinal analysis by diagnostic group is based on baseline diagnosis.

3.2. Longitudinal clinical change over time

Mean (SD) CDR sum of boxes increased significantly over 12 months in the AD group [from 5.9 (2.4) to 7.3 (2.9), $P = .002$], but not the NC or MCI groups (see Table 1). Mini-Mental State Examination (MMSE) scores showed little change in NC and MCI groups, but decreased significantly in the AD group over the 12-month interval [from 22.4 (3.1) to 19.2 (4.9), $P < .001$]. Compared with the NC group, the AD group declined more on the MMSE between 0 and 6 months ($\chi^2 = 11.41$, $P = .003$) and 0 and 12 months ($\chi^2 = 21.23$, $P < .001$). There were also significant within-group changes in the cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-cog) in the AD group ($\delta = 4.70$, $P = .002$) and a between-group change over 12 months between the AD and NC groups ($\chi^2 = 11.40$, $P = .010$).

3.3. Region-specific cross-sectional group comparison of FA and MD

MD values for the fornix (FX1 and FX2) and splenium (SP) were consistently higher in the AD group at all time-points when compared with both the NC and MCI groups (Table 2 and Fig. 1). At baseline, the AD group also had higher mean MD values in both slices of the posterior cingulum (CG2: 0.260 vs 0.242, $P = .006$; CG3: 0.257 vs 0.240; $P = .005$) compared with the MCI group. At the 6-month visit, the AD group, compared with the NC group, also had higher mean MD in the cerebral peduncles (0.283 vs 0.277, $P = .007$) and inferior cingulum (0.249 vs 0.226, $P = .002$). There were no differences between the NC and MCI groups in any region at the $P < .01$ level. FA of the FX1 and FX2 and SP were consistently lower in the AD group compared with both the NC and MCI groups at all time-points (Table 3). However, only comparisons between the NC and AD groups in the fornix and splenium reached statistical significance at $\alpha = .01$. Mean FA did not vary between NC and MCI in any region.

3.4. Within- and between-group change over time: FA and MD

In general, changes of FA and MD between successive visits were very small within groups (Tables 2 and 3). Consistent changes in FA or MD were not observed. There were significant within- and between-group changes in MD (Table 4 and Fig. 2). Within groups, among NC, MD decreased over 12 months in both slices of the posterior cingulum (CG2: $\delta = -0.11$, $P = .002$; CG3: $\delta = -0.10$; $P = .008$), but increased in the inferior cingulum (CG1: $\delta = 0.25$, $P < .001$) and fornix (FX2: $\delta = 0.40$, $P = .010$). In the MCI group, there were 12-month MD increases in the inferior cingulum (CG1: $\delta = 0.25$, $P < .001$), anterior cingulum

(CG4: $\delta = 0.13$, $P = .009$), and both slices of the fornix (FX1: $\delta = 0.51$, $P < .001$; FX2: $\delta = 0.52$; $P < .001$). In the AD group, there were 12-month MD increases in the inferior cingulum (CG1: $\delta = 0.21$, $P = .007$) and in one slice of the fornix (FX2: $\delta = 0.63$, $P = .003$).

Regarding between-group MD changes, compared with NC, both the MCI and AD groups had greater MD increases in the inferior cingulum (CG1: NC vs MCI: $\chi^2 = 10.29$, $P = 0.006$; NC vs AD: $\chi^2 = 10.59$; $P = .005$) over 6 months. Over 12 months, these differences with NCs were notable for both the MCI ($P = .016$) and AD ($P = .016$) groups. Compared with the NC group, the MCI group also had a greater increase in one area of the fornix MD over both 6 months (FX1: $\chi^2 = 10.36$, $P = .006$) and 12 months (FX1: $\chi^2 = 12.03$, $P = .007$).

FA changes were noted only in the NC, among whom it increased significantly over the 12-month period in the posterior cingulum (CG2: $\delta = 0.036$, $P < .001$) and splenium ($\delta = 0.022$, $P = .007$). Although there was decreased FX2 FA in the NC group over 12 months (FX2: $\delta = -0.021$, $P = .027$), this change was not significant at the $\alpha = 0.01$ level. FA did not change between 0 and 6 or 0 and 12 months in either the MCI or AD groups. There were also no between-group differences in FA change over 6 or 12 months.

3.5. Subanalysis: “Converter” dropout effect (data not shown)

We conducted a subanalysis to see if change in diagnostic status or dropout would have an effect on cross-sectional and longitudinal DTI variables or clinical measures. At 12 months, of the 25 NC participants, 1 was diagnosed as “cognitively impaired” but not demented, and 2 were diagnosed with MCI. Of the 25 MCI participants, 3 converted to AD, whereas 2 nonconverters were lost to follow-up at their last visit. Of 25 AD participants, 2 were lost to follow-up by 12 months. Exclusion of converters and dropouts from the analyses did not result in a significant change in DTI variables or aggregate cognitive measures at cross-section or longitudinally.

4. Discussion

In this DTI study of NC, MCI, and AD participants, we examined WM differences (FA and MD) four times over 12 months within and between diagnostic groups in eight *a priori*-defined ROIs. There were stable, region-specific, cross-sectional differences at all time-points between the NC and AD groups for FA and MD in both slices of the fornix and the splenium. However, increasing MD in the fornix was evident in the MCI group only. Further, fornix MD increased faster over the 12-month period in MCI cases compared with NC. In contrast, there were no notable within- or between-group FA changes over time.

DTI is a noninvasive technique used to measure white-matter integrity by imaging the anisotropic motion of water molecules. FA and MD report scalar values that approximate fiber integrity, generally in areas of highly concentrated axons. Reductions in FA and increases in MD reflect less constrained motion of water molecules about the longitudinal axis of axons. Decreasing FA is thought to represent changes in tissue cytoarchitecture due to a variety of causes, including vascular alteration, demyelination, and possibly gliosis [39, 40]. Increasing MD most likely results in an increase in extracellular space and elevated water diffusivity in all directions and probably represents loss of neurons, axons, and dendrites in neurodegenerative diseases [41, 42]. In patients with AD, it is believed that WM degradation is a result of Wallerian degeneration and rarefaction [12, 40]. These may be a part of a cortical disconnection that affects, in a progressive fashion, the cortical neuronal soma as well as axons and dendrites in the cerebral WM [21, 43–45]. Postulated causes of the resultant inhomogeneity of WM microstructure include: myelin pallor; progressive demyelination; axonal or dendritic degeneration; complete invasion and deposition of A β

peptide; and replacement of the neuronal soma by neurofibrillary tangles [46–48]. The precise pathogenetic mechanisms for these WM changes remain unclear.

We observed significantly increasing MD (inferior cingulum, anterior cingulum, and fornix) within the MCI group over the 12-month study period, even though clinical changes were not evident. These findings are in partial agreement with the only other longitudinal DTI study published to date (Teipel et al [30]), which demonstrated significant change in a clinically stable MCI group. Unlike their study, we showed significant changes in MD, not FA. Further, we found that both the fornix and cingulum changed significantly, whereas Teipel et al reported change in the corpus callosum. Despite these discrepancies, both studies showed a striking signal within the MCI group, even when there was little cognitive change. Although the precise neural correlates of altered diffusivity are not completely understood, our findings suggest that MD is a more specific indicator of longitudinal change in MCI that likely precedes clinical change. This is consistent with the view (see earlier) that MD is more sensitive to neurodegeneration.

Our stable cross-sectional findings of lower FA and higher MD, primarily in the fornix and splenium, are consistent with many other studies in AD demonstrating impaired WM integrity in similarly investigated ROIs [14, 16, 18, 21, 23, 25, 28, 30, 49–51]. Although we also saw significant group differences in other areas (e.g., the mean MD in inferior cingulum [CG1] between NC and MCI at the 6-month time-point), the latter were not consistent and are most likely due to chance. In contrast, differences in the fornix and splenium were consistent across all time-points when comparing the NC and AD groups, suggesting these findings are stable. Notably, there were few cross-sectional differences between the MCI and NC groups or the MCI and AD groups, suggesting that these measures cannot be used for clinical diagnosis to distinguish between these groups.

The absence of predictable and consistent within-group longitudinal change in AD patients over the 12 months is unexpected, especially considering the significant clinical decline in the AD group. We initially hypothesized that we would find decreasing FA or increasing MD among AD patients. Our results indicate that WM changes detectable by current DTI methods, even when considering the potential for measurement error, occurred early in the Alzheimer's disease process, during the MCI phase. WM changes in the AD group may have reached a maximal level and plateaued, even when clinical change continued to progress. Current understanding of the spread of AD pathology as starting in the medial temporal lobe and spreading to other heteromodal association cortices [52] supports our observations that the “spread” took place in the areas of WM that we studied and likely other areas as well. Our assertion that the greatest change occurs in the prodromal stage of AD is consistent with the current understanding that pathologic alterations precede clinical presentation.

Notably, there were small increases and decreases of FA and MD in all groups between baseline and 6 months. However, these changes seem too small to effectively represent meaningful anatomic change, especially when considering measurement variance at cross-sectional time-points. As such, we suggest that increases in FA or decreases in MD in any diagnostic group over a short time frame (i.e., 0–6 months) should not be interpreted as a marker of anatomic change, especially if there are no clinical changes. In contrast, changes over 6 months, especially in MCI, may be of value as biomarkers of the effects of AD on the WM. We caution against making overly generalized assumptions about using DTI as a longitudinal measure before these results are replicated.

Finally, we consider the fornix as an area of increasing importance in the pathobiology of AD. These findings (see Table 4 and Fig. 2) further suggest that the fornix is affected mostly

during the MCI stage, or earlier, and that as we hypothesized before, early degeneration of the fornix in MCI might be an early indicator of progression. It also follows that the fornix would be implicated in the progressive decline of AD because it serves as cholinergic input into the hippocampus as well as performing outflow projections to other areas in the brain [53]. The disconnection syndrome referred to previously fits well with the secondary degeneration model of AD where the loss of WM integrity continues to represent downstream effects of neuronal pathology [46, 52, 54]. The specific cause of the pathology, whether by primary damage to myelin or by axonal damage, continues to be debated.

Our study is not without limitations. We acknowledge that ROI methods may not be optimal in studying longitudinal changes in MCI and AD. ROI methods of DTI analysis are subject to *a priori* assumptions of regions of pathologic damage and are operator-dependent. Atlas-based methods are promising approaches for examining larger numbers of regions without the constraints of abiding by theoretical hypotheses [55, 56]. We note, however, that atlas-based analyses of this data set are consistent with our ROI findings with regard to the importance of the fornix. Future studies may include voxel-by-voxel or atlas-based analyses to take into account additional anatomic regions [57]. Other studies may also compare and contrast longitudinal measurements of different imaging modalities, such as positron emission tomography (PET), MRI morphometry, etc., to DTI. More work is needed to replicate our findings, test other regions, as well as to track changes at intervals of longer than 12 months.

Our study also has several strengths. First, to our knowledge, this is one of the first studies to serially examine DTI cross-sectionally, and longitudinally over 1 year in both MCI and AD. This is important because MCI is thought to be the prodromal stage of AD and understanding the anatomic changes of WM as sequential stages corresponding to clinical diagnosis is important for understanding the inferred pathophysiology of the disease. Second, although 25 subjects per study group is a low number, our sample size represents one of the larger DTI cohorts in the MCI and AD literature. Third, we examined both FA and MD. This is significant because our results suggest that MD may be more sensitive to change over a 12-month period.

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Fig. 1.
Mean Diffusivity (MD) between-group differences: Fornix (FX1 and FX2), Splenium (SP) at baseline (v1) and 12 months (v4).

Fig. 2.
Mean Diffusivity (MD) by baseline diagnostic group: Alzheimer's Dementia (AD), Mild Cognitive Impairment (MCI), Normal Control (NC) at 0, 3, 6, 12 month time points.

Table 1

Participant demographics and clinical changes

	NC (SD) or n (%)		MCI (SD) or n (%)		AD (SD) or n (%)	
	1	4	1	4	1	4
Visit						
n	25	23	25	23	25	23
Mean (SD), years	74.3 (7.1)	75.3 (7.1)	75.8 (5.3)	76.7 (5.1)	75.6 (7.0)	76.1 (6.8)
Female	14 (56.0%)	14 (63%)	7 (28.0%)	7 (32%)	7 (28.0%)	5 (19%)
Education	16.2 (2.6)	16.2 (2.6)	15.7 (3.0)	15.6 (3.1)	14.4 (3.9)	15 (4.0)
CDR-SB	0.02 (0.1)	0.10 (0.3)	1.3 (0.79)	1.4 (1.2)	5.9 (2.4)	7.3 (2.9)
CDR-SB (<i>P</i>)	.1		.67		.002	
MMSE	28.8 (1.2)	29.1 (1.0)	26.6 (2.1)	26.8 (2.7)	22.4 (3.1)	19.2 (4.9)
MMSE (<i>P</i>)	.25		.69		<.001	

Abbreviations: NC, normal controls; MCI, mild cognitive impairment; AD, Alzheimer’s disease; MMSE, Mini-Mental State Examination; CDR-SB, Clinical Dementia Rating-Sum of Boxes.

Table 2

Cross-sectional mean diffusivity (MD) on images at visits 1–4 (between-group comparison)

ROI	Visit	NC e^{-2} (SD e^{-2})	MCI e^{-2} (SD e^{-2})	AD e^{-2} (SD e^{-2})	NC vs AD (<i>P</i> -value)	MCI vs NC (<i>P</i> -value)	MCI vs AD (<i>P</i> -value)
CP	1	0.276 (0.023)	0.264 (0.049)	0.279 (0.021)	.622	.286	.184
	2	0.282 (0.050)	0.261 (0.051)	0.278 (0.024)	.738	.154	.166
	3	0.282 (0.050)	0.277 (0.024)	0.283 (0.022)	.007	.074	.370
	4	0.282 (0.023)	0.277 (0.022)	0.288 (0.029)	.428	.361	.124
CG1	1	0.229 (0.027)	0.231 (0.029)	0.246 (0.033)	.045	.757	.094
	2	0.233 (0.020)	0.228 (0.032)	0.236 (0.024)	.593	.488	.298
	3	0.226 (0.020)	0.245 (0.035)	0.249 (0.026)	.002	.024	.670
	4	0.255 (0.028)	0.257 (0.026)	0.267 (0.036)	.205	.777	.302
CG2	1	0.254 (0.019)	0.242 (0.021)	0.260 (0.023)	.321	.039	.006
	2	0.252 (0.017)	0.245 (0.031)	0.259 (0.017)	.155	.295	.051
	3	0.246 (0.015)	0.244 (0.015)	0.256 (0.021)	.081	.560	.031
	4	0.243 (0.015)	0.248 (0.031)	0.253 (0.013)	.022	.461	.519
CG3	1	0.247 (0.018)	0.240 (0.018)	0.257 (0.023)	.072	.209	.005
	2	0.246 (0.017)	0.238 (0.030)	0.254 (0.018)	.132	.226	.029
	3	0.241 (0.015)	0.240 (0.014)	0.248 (0.016)	.162	.716	.087
	4	0.237 (0.016)	0.238 (0.020)	0.247 (0.015)	.024	.905	.068
CG4	1	0.244 (0.018)	0.239 (0.031)	0.255 (0.027)	.121	.478	.071
	2	0.238 (0.022)	0.238 (0.027)	0.244 (0.023)	.348	.960	.376
	3	0.241 (0.019)	0.244 (0.019)	0.257 (0.026)	.021	.678	.049
	4	0.249 (0.022)	0.251 (0.018)	0.253 (0.060)	.606	.838	.714
FX1	1	0.351 (0.070)	0.346 (0.081)	0.421 (0.099)	.006	.787	.005
	2	0.348 (0.064)	0.357 (0.101)	0.401 (0.012)	.064	.704	.189
	3	0.346 (0.071)	0.383 (0.077)	0.413 (0.012)	.028	.090	.336
	4	0.354 (0.084)	0.386 (0.080)	0.447 (0.012)	.004	.188	.054
FX2	1	0.401 (0.074)	0.411 (0.078)	0.475 (0.011)	.011	.645	.029
	2	0.391 (0.066)	0.421 (0.098)	0.465 (0.012)	.011	.213	.177
	3	0.408 (0.074)	0.454 (0.075)	0.498 (0.013)	.007	.043	.181
	4	0.441 (0.094)	0.456 (0.084)	0.532 (0.011)	.004	.565	.014

ROI	Visit	NC e ⁻² (SD e ⁻²)	MCI e ⁻² (SD e ⁻²)	AD e ⁻² (SD e ⁻²)	NC vs AD (P-value)	MCI vs NC (P-value)	MCI vs AD (P-value)
SP	1	0.297 (0.026)	0.296 (0.037)	0.319 (0.030)	.011	.881	.024
	2	0.301 (0.020)	0.299 (0.042)	0.317 (0.034)	.031	.978	.111
	3	0.287 (0.032)	0.304 (0.026)	0.312 (0.023)	.004	.051	.253
	4	0.300 (0.023)	0.314 (0.030)	0.324 (0.026)	.002	.076	.233

Abbreviations: CP, cerebral peduncles; CG1–4, cingulum bundle, regions 1–4; FX1–2, fornix, slices 1–2; SP, splenium. Bold P-values are statistically significant.

Table 3
Cross-sectional mean fractional anisotropy (FA) on images at visits 1–4 (between-group comparison)

ROI	Visit	NC (SD)	MCI (SD)	AD (SD)	NC vs AD (P-value)	MCI vs NC (P-value)	MCI vs AD (P-value)
CP	1	0.610 (0.043)	0.625 (0.035)	0.606 (0.047)	.751	.173	.173
	2	0.606 (0.045)	0.616 (0.043)	0.621 (0.042)	.208	.415	.645
	3	0.621 (0.049)	0.627 (0.048)	0.625 (0.041)	.773	.689	.897
	4	0.624 (0.035)	0.629 (0.045)	0.622 (0.065)	.868	.646	.635
CG1	1	0.414 (0.046)	0.406 (0.040)	0.397 (0.031)	.124	.514	.345
	2	0.406 (0.035)	0.406 (0.033)	0.397 (0.039)	.369	.970	.381
	3	0.420 (0.034)	0.406 (0.037)	0.394 (0.036)	.018	.186	.301
	4	0.416 (0.041)	0.413 (0.036)	0.407 (0.055)	.495	.737	.671
CG2	1	0.444 (0.040)	0.449 (0.047)	0.448 (0.053)	.732	.654	.948
	2	0.452 (0.041)	0.443 (0.045)	0.445 (0.045)	.585	.471	.874
	3	0.446 (0.047)	0.454 (0.053)	0.453 (0.052)	.644	.959	.949
	4	0.479 (0.028)	0.475 (0.063)	0.467 (0.050)	.310	.776	.642
CG3	1	0.456 (0.042)	0.468 (0.037)	0.455 (0.052)	.943	.314	.346
	2	0.455 (0.037)	0.456 (0.035)	0.450 (0.047)	.682	.921	.622
	3	0.456 (0.039)	0.467 (0.039)	0.465 (0.044)	.484	.335	.848
	4	0.482 (0.046)	0.492 (0.064)	0.475 (0.053)	.597	.560	.334
CG4	1	0.439 (0.040)	0.443 (0.050)	0.408 (0.062)	.040	.758	.033
	2	0.446 (0.039)	0.428 (0.054)	0.412 (0.052)	.014	.197	.306
	3	0.437 (0.039)	0.437 (0.040)	0.407 (0.056)	.040	.995	.046
	4	0.452 (0.051)	0.439 (0.044)	0.420 (0.058)	.045	.358	.204
FX1	1	0.512 (0.060)	0.503 (0.084)	0.438 (0.100)	.003	.659	.018
	2	0.515 (0.050)	0.493 (0.090)	0.463 (0.116)	.046	.288	.327
	3	0.521 (0.061)	0.501 (0.068)	0.466 (0.084)	.016	.305	.134
	4	0.519 (0.063)	0.492 (0.072)	0.440 (0.104)	.002	.179	.053
FX2	1	0.499 (0.048)	0.478 (0.066)	0.422 (0.088)	.0004	.193	.016
	2	0.495 (0.062)	0.463 (0.075)	0.436 (0.093)	.012	.117	.266
	3	0.488 (0.063)	0.463 (0.053)	0.421 (0.098)	.008	.142	.083
	4	0.479 (0.069)	0.462 (0.072)	0.405 (0.084)	.002	.409	.018
SP	1	0.636 (0.050)	0.632 (0.041)	0.605 (0.045)	.031	.758	.035

ROI	Visit	NC (SD)	MCI (SD)	AD (SD)	NC vs AD (<i>P</i> -value)	MCI vs NC (<i>P</i> -value)	MCI vs AD (<i>P</i> -value)
	2	0.630 (0.035)	0.625 (0.037)	0.604 (0.049)	.040	.660	.097
	3	0.648 (0.053)	0.636 (0.037)	0.622 (0.049)	.096	.363	.298
	4	0.655 (0.041)	0.629 (0.051)	0.619 (0.049)	.007	.056	.485

NOTE. Refer to Table 2 for abbreviations. Bold *P*-values are statistically significant.

Table 4

Mean diffusivity (MD) longitudinal within- and between-group comparisons from 0 to 6 and 0 to 12 months

ROI	Within-group comparison				Between-group comparison			
	NC		MCI		AD		MCI vs NC	
	δ	P -value	δ	P -value	δ	P -value	χ^2	P -value
CP	0-6	-0.012	.006	0.012	.216	0.004	.533	.023
	0-12	0.006	.183	0.012	.292	0.009	.230	.011
CG1	0-6	-0.003	.553	0.013	.066	0.003	.654	.006
	0-12	0.025	<.001	0.025	<.001	0.021	.007	10.29
CG2	0-6	-0.008	.004	0.002	.768	-0.005	.297	.279
	0-12	-0.011	.002	0.005	.454	-0.008	.144	.225
CG3	0-6	-0.005	.070	-0.000	.908	-0.011	.007	.488
	0-12	-0.010	.008	-0.003	.560	-0.011	.013	.492
CG4	0-6	-0.005	.224	0.006	.406	0.003	.617	.404
	0-12	0.004	.241	0.013	.009	-0.002	.713	.482
FX1	0-6	-0.007	.439	0.048	.001	-0.004	.761	10.36
	0-12	0.003	.788	0.051	<.001	0.030	.047	12.03
FX2	0-6	0.006	.613	0.041	.006	0.025	.207	.193
	0-12	0.040	.010	0.052	<.001	0.063	.003	.425
SP	0-6	-0.012	.005	0.008	.386	-0.004	.443	.072
	0-12	0.001	.839	0.018	.017	0.005	.377	.070

NOTE. Refer to Table 2 for abbreviations. Bold P -values are statistically significant.

* δ represents fitted mean changes over time.