# White Matter Microstructural Change Contributes to Worse Cognitive Function in Patients With Type 2 Diabetes

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**Patients with type 2 diabetes mellitus (T2DM) have a considerably high risk of developing dementia, especially for those with mild cognitive impairment (MCI). The investigation of the microstructural change of white matter (WM) between T2DM with amnesic MCI (T2DM-aMCI) and T2DM with normal cognition (T2DM-NC) and their relationships to cognitive performances can help to understand the brain variations in T2DM-related amnesic cognitive impairment. In the current study, 36 T2DM-aMCI patients, 40 T2DM-NC patients, and 40 healthy control (HC) individuals underwent diffusion tensor image and T1-weighted MRI scans and comprehensive cognition assessments. All of these cognitive functions exhibited intergroup ranking differences in patients. The T2DM-NC patients and HC individuals did not reveal any significant differences in WM integrity. The T2DM-aMCI patients showed disrupted integrity in multiple WM tracts compared with HC and T2DM-NC. Specifically, the damaged WM integrity of the right inferior fronto-occipital fasciculus and the right inferior longitudinal fasciculus exhibited significant correlations with episodic memory and attention function impairment in T2DM patients. Furthermore, cognitive impairment-related WM microstructural damage was associated with the degeneration of cortex connected to the affected WM tract. These findings indicate that degeneration exists extensively in WM tracts in T2DM-aMCI, whereas no brain WM damage is evident in T2DM-NC.**

Diabetes is a common metabolic disorder that can lead to chronic complications such as cardiovascular disease and peripheral neuropathy. In diabetes, the type 2 diabetes mellitus (T2DM) caused by insulin resistance accounts for 90–95% of diabetes cases. Previous reports have indicated that T2DM is associated with an increase in the risk of dementia and in the proportion of patients who convert from mild cognitive impairment (MCI) to dementia (1). T2DM is associated with amnestic MCI (aMCI) and non-amnestic MCI (2,3), the former being considered to be a prodromal stage of Alzheimer disease (AD) (4). The evidence of a link between T2DM and aMCI seems relatively less and weak and is the subject of much debate.

Recent studies demonstrated a pathophysiological link between T2DM and cognitive impairment as well as the underlying pathways that connect these two diseases (5). Longitudinal population-based studies have revealed that the relative risk of dementia is 1.2- to 2.8-times higher in individuals with diabetes relative to those without diabetes (6). In addition, different stages of diabetes-associated cognitive dysfunction exist with different cognitive features (7,8), and the rates of conversion to dementia in patients with T2DM with MCI (T2DM-MCI) are significantly higher compared with T2DM patients who were cognitively intact and with MCI patients without diabetes (9). The mean annual conversion rate to dementia of the T2DM-MCI and cognitively intact T2DM cohorts, respectively, was 8.43% and 1.64% per year and was 3.86% per year for the MCI cohort without T2DM (9), which suggests that more brain toxicity may have occurred in T2DM with cognitive impairment than MCI without T2DM or T2DM with normal cognition (T2DM-NC). However, it is still

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unclear, and limited literature has researched, why a subset of T2DM patients develop MCI and how they are different, brainwise, from non-MCI patients.

As reported previously, many studies have revealed that white matter (WM) integrity measured by a diffusion tensor imaging (DTI) technique is particularly sensitive to T2DM (10–13) and cognitive impairment (14). These studies currently showed that regions of WM integrity degeneration caused by T2DM are scattered and inconsistent across the entire brain. Reijmer et al. (12), for example, found WM integrity damage in the superior longitudinal fasciculus (SLF), the inferior longitudinal fasciculus (ILF), and the uncinate fasciculus (UF) in T2DM patients. Hoogenboom et al. (11) demonstrated that patients with T2DM exhibited lower integrity in the cingulum bundle and the UF, whereas patients did not show significantly lower integrity in the SLF. Zhang et al. (10) revealed that T2DM patients with various degrees of cognitive impairment exhibited widespread WM disruptions, especially in the whole corpus callosum, the left anterior limb of the internal capsule, and external capsule. These inconsistent results may occur because T2DM patients were in different stages of diabetes-associated cognitive dysfunction in different studies. Therefore, this study investigated T2DM patients with and without risk of aMCI and the WM integrity mechanism.

In the current study, we enrolled T2DM-aMCI patients, T2DM-NC patients, and healthy control (HC) subjects to conduct clinical assessments, a battery of neuropsychological tests, DTI, and T1-weighted imaging. Voxel-wise analyses were applied to investigate WM differences in the three groups, and then we researched the relationships between WM injuries and cognitive performance to test for cognitive impairment-related WM fibers in T2DM. Finally, we evaluated whether cognitive impairment-related WM microstructural damage was associated with the degeneration of cortex connected to the affected WM tract. We expected that our study could provide insight into the pathogenic mechanisms of T2DM-related cognitive impairment.

## RESEARCH DESIGN AND METHODS

### Participants

All participants of this study were recruited from the Beijing Aging Brain Rejuvenation Initiative (BABRI) project, an ongoing longitudinal study from an urban population in Beijing, China. There are 36 T2DM-aMCI, 40 T2DM-NC, and 40 HC subjects. The participant inclusion criteria were: *1*) no less than 6 years of education; *2*) aged between 50 and 80 years; *3*) right-handed; *4*) Mini-Mental State Examination (MMSE) scores >24 on the Chinese version; *5*) no history of coronary disease, nephritis, tumors, gastrointestinal disease, or psychiatric illness; *6*) no contraindications to MRI; and *7*) no obvious microvascular disease. All participants self-reported to have never experienced severe hypoglycemia and to have a low frequency of minor hypoglycemia. The Beijing Normal University Institutional Review Board approved the study. Each participant provided written informed consent.

All T2DM patients had a history of using oral antidiabetic medications, insulin, or no treatment and were diagnosed using established criteria based on the patients' medical histories, medication use, or fasting plasma glucose (FPG) levels (>7 mmol/L). Of the 36 T2DM-aMCI patients, 9 were being treated with insulin, 4 were being treated with diet and exercise, and 23 controlled their blood glucose with oral hypoglycemic agents (6 with glucosidase inhibitors, 11 with biguanides, 3 with sulfonylurea, 1 with nateglinide, and 2 with glucosidase inhibitors and biguanides). Of 40 T2DM-NC patients, 5 were receiving treatment with insulin, 11 were being treated with diet and exercise, and 24 controlled their blood glucose with oral hypoglycemic agents (2 with glucosidase inhibitors, 5 with biguanides, 2 with sulfonylurea, 12 with 2 drugs combined, and 3 with 3 drugs combined). BMI, FPG, glycosylated hemoglobin ($HbA_{1c}$), triglyceride, total cholesterol, HDL, and LDL levels were measured via standard laboratory testing.

### Neuropsychological Testing

All participants completed a battery of neuropsychological tests, including the MMSE (15) and the following six cognition domains and associated tests:

1. Episodic memory: Auditory Verbal Learning Test (AVLT)-Delay Recall and AVLT-Total (16), and Rey-Osterrieth Complex Figure (ROCF)-Delay Recall test (17)
2. Working memory: the digit span test and digit span backward (18)
3. Spatial processing: ROCF-Copy (17) and Clock Drawing Test (19)
4. Executive function: Trail Making Test (TMT)-B and Stroop Color and Word Test–C-B (20)
5. Language ability: Category Verbal Fluency Test and Boston Naming Test (21)
6. Attention: TMT-A and Symbol Digit Modalities Test (20)

We diagnosed aMCI subjects according to Petersen (22) criteria for aMCI, including subjective memory complaints, cognitive impairment in memory (scoring >1.5 SDs below the age- and education-adjusted norm on at least two tests with the AVLT-Delay Recall, AVLT-Total or ROCF-Delay Recall), normal general cognitive function (scoring no less than 24 on the MMSE), essentially intact activities of daily living, and lack of dementia. If memory was the only area of impairment, the subject was considered to be single-domain aMCI. If memory plus other cognitive domains assessed with neuropsychological testing were affected, the subject was considered to be multidomain aMCI. The study included single-domain aMCI and multidomain aMCI. The HC subjects had no evidence of cognitive deficits on any of these the neuropsychological tests.

### MRI Data Acquisition

Imaging data were collected using a Siemens Trio 3-T MRI system including DTI data and T1-weighted image scans.

Participants were positioned supine with their head snugly fixed by straps and foam pads to minimize head movement. Diffusion-weighted imaging was performed using a single-shot, twice-refocused, diffusion-weighted echo planar sequence aligned along the anterior-posterior commissural plane. T1-weighted, sagittal three-dimensional magnetization prepared rapid gradient echo (MP-RAGE) sequences were acquired and covered the entire brain. The parameters of DTI and T1-weighted images refer to our previous study (23).

### DTI Data Preprocessing and Analysis

The FMRIB Diffusion Toolbox (FDT, v3.0) in the FMRIB Software Library (FSL, v5.0.10) (24) was used for image preprocessing. Briefly, the preprocessing involves correction of eddy current and head movement, creating a brain mask and fitting the diffusion tensor model. The output yielded voxel-wise maps of fractional anisotropy (FA), mean diffusivity, radial diffusivity, and axial diffusivity. The FA index of DTI is the most sensitive neuroimaging measure of the degeneration observed in T2DM patients and describes overall WM health, maturation, and organization (25). Another index, axial diffusivity, reflects axon integrity and can be useful in understanding the underlying physiology (26). Voxel-wise statistical analysis of the FA data was performed using Tract-Based Spatial Statistics (TBSS) (27), part of FSL (24). TBSS projects all subjects' FA data onto a mean FA tract skeleton before applying voxel-wise cross-subject statistics.

Voxel-wise statistical analyses were performed using a nonparametric permutation-based inference tool ("randomize," part of FSL) with the general linear model (GLM) as statistical modeling. First, pairwise group comparisons based on voxels were performed using GLM for T2DM-aMCI versus HC, T2DM-aMCI versus T2DM-NC, and T2DM-NC versus HC. The DTI parameters at each voxel were modeled as a linear combination of predictors (three grouping variables) and covariates (sex, age, education, hypertension, hyperlipidemia, and cerebrovascular disease) stored in the columns of a "design matrix." For subsequent analyses, we calculated WM volumes in regions with between-group differences, and DTI parameters at voxels within significant intergroup different tracts in all T2DM patients. WM tracts were identified using the JHU_ICBM_tracts_maxprob_thr25 atlas (28).

Second, a voxel-wise linear relationship was determined using GLM in all T2DM. The DTI parameters at each voxel in significant intergroup different tracts are modeled as a linear combination of cognitive scores with significant intergroup difference domains and covariates. The significance threshold for between-group differences and linear relationships were set at $P < 0.05$ (5,000 permutations, familywise error [FWE] correction for multiple comparisons correction) using the threshold-free cluster enhancement.

Next, we extracted the mean value of axial diffusivity on significantly related fibers for subsequent analyses. FA of significant intergroup different tracts did not show any significant relationships with cognitive measures.

### T1 MRI Data Processing and Analysis

T1-weighted images were analyzed with the FSL voxel-based morphometry (FSLVBM) pipeline (24,29). First, structural images were brain extracted and gray matter segmented before being registered to the Montreal Neurological Institute (MNI) 152 standard space using nonlinear registration. The resulting images were averaged and flipped along the *x*-axis to create a left-right symmetric, study-specific gray matter template.

Second, all native gray matter images were nonlinearly registered to this study-specific template and "modulated" to correct for local expansion due to the nonlinear component of the spatial transformation. The modulated gray matter images were then smoothed with an isotropic Gaussian kernel with a $\sigma$ of 3 mm. All the modulated registered gray matter images were concatenated into a four-dimensional image, and the file was fed into voxel-wise statistics.

Next, a voxel-wise linear relationship was determined with GLM in all T2DM using a randomize tool to evaluate whether cognitive impairment-related WM microstructural damage was associated with the degeneration of cortex connected to the affected WM tract. The gray matter density in regions of interests (ROIs) at each voxel was modeled as a linear regression of diffusion metrics in cognitive impairment-related WM tracts and covariates (sex, age, education, hypertension, hyperlipidemia, and cerebrovascular disease). The significance threshold for a linear relationship was set at $P < 0.05$ (5,000 permutations, FWE-correction for multiple comparisons). In this study, the gray matter ROIs were the lingual and frontal middle orbital (Frontal_Mid_Orb) with anatomic connections to inferior fronto-occipital fasciculus (IFOF) as well as the temporal inferior (Temporal_Inf) and occipital middle (Occipital_Mid) with anatomic connections to ILF (30).

### Statistical Analysis

Consistency in age, sex, and education level among the T2DM-aMCI, T2DM-NC, and HC groups was determined with one-way ANOVA or $\chi^2$ analysis. The neuropsychological assessment and clinical data were analyzed using an ANCOVA, with age, sex, education, hypertension, hyperlipidemia, and cerebrovascular disease included as covariates. Post hoc pairwise *t* tests with Bonferroni correction were performed to follow the significant main effects yielded by the ANOVA and ANCOVA tests ($P < 0.05$). To adjust for possible spurious findings due to multiple ANCOVA testing, Bonferroni correction was conducted for all the cognition tests. Next, we assessed the relationship between the mean value of axial diffusivity of the significant region (SR, cognitive scores significantly related to the WM index in this region) and the neuropsychological scores in all T2DM patients, which was performed with the regression correlation analysis. Similarly, we assessed

the regression correlation between cognitive impairment–related diffusion metrics in the SRs and gray matter density in all T2DM patients. Regression correlations were performed after controlling for the influences of age, sex, and education (Bonferroni correction for multiple WM fibers or multiple gray matter regions). We present normalized cognitive scores as well as *z* values. To test which cognitive functions were most affected, we calculated the percentage of patients with a score <1.5 SD across all participants in Supplementary Data (Supplementary Table 1). All statistical analyses were performed using SPSS 22.0 software. Please see Supplementary Data for the technical MRI details.

### Data and Resource Availability

The data sets generated during and/or analyzed during the current study are not publicly available because they are under construction but are available from the corresponding author upon reasonable request. No applicable resources were generated or analyzed during the current study.

## RESULTS

### Demographics and Neuropsychological Testing

Demographic data and neuropsychological testing are presented in Table 1 and Supplementary Fig. 1. There were no significant differences in age, sex, year of education, BMI, total cholesterol, triglycerides, HDL, or LDL among the T2DM-aMCI, T2DM-NC, and HC groups. There was no difference in T2DM disease duration between T2DM-aMCI and T2DM-NC, but significant differences of $HbA_{1c}$ were observed ($P < 0.001$) between the HC group and each of the T2DM groups. As expected, the MMSE scores were significantly higher in HC subjects compared with T2DM-aMCI and T2DM-NC patients, and T2DM-NC patients performed better than T2DM-aMCI patients. For various neuropsychological scores, one-way ANOVA showed that there were significant group effects for most of the cognitive domains with the best performance in HC subjects, an intermediate performance in T2DM-NC patients, and the worst performance in T2DM-aMCI patients. Comparing the neuropsychological test scores between the T2DM-aMCI and T2DM-NC groups revealed significant differences in the episodic memory, working memory, spatial processing, language ability, and attention domains; however, there were no differences in executive function.

### Group Differences in WM Diffusion Metrics Among the Groups

We assessed voxel-wise WM integrity differences with DTI parameters between each group pair (Fig. 1 and Supplementary Table 2). Compared with the HC subjects, T2DM-aMCI patients showed a significantly decreased FA value in widespread WM tracts, including the bilateral anterior thalamic radiation (ATR), bilateral corticospinal tract (CST), bilateral cingulum, forceps minor, bilateral IFOF, bilateral SLF, bilateral UF, and left SLF temporal (SLF-temp) part (FWE correction, $P < 0.05$). Compared with T2DM-NC patients, T2DM-aMCI patients showed significantly reduced FA value decreases in right ATR, right CST, forceps major, forceps minor, right IFOF, right ILF, right SLF, and right SLF-temp (FWE correction, $P < 0.05$). There were no significant differences between T2DM-NC patients and HC subjects. Volumes in intergroup differences in WM regions were calculated and are reported in Table 2. Axial and mean diffusivity of WM regions showed no significant difference among the three groups.

### WM Integrity in Relation to Cognitive Performance

Based on the WM tracts with significant intergroup differences (FWE correction, $P < 0.05$), we explored the relationship between diffusion metrics (FA and axial diffusivity) of those tracts as well as the neuropsychological scores in all T2DM patients. The results of correlation analysis based on voxel-wise show that the TMT-A test was significantly correlated with the axial diffusivity of ATR.right hemisphere (R), IFOF.R, ILF.R, SLF.R, SLF-temp.R, UF.R, CST.R, and CST.left hemisphere (L), and AVLT-Total was significantly correlated with the axial diffusivity of forceps major, IFOF.R and ILF.R. The results of regression correlations based on tractwise showed higher axial diffusivity for the forceps major ($\beta = 0.359$, $P = 0.004$), the IFOF.R ($\beta = 0.441$, $P < 0.001$, in SR 1), and the ILF.R ($\beta = 0.431$, $P < 0.001$) in SR 4, which was significantly correlated with better episodic memory performance (AVLT-Total) after Bonferroni correction (Fig. 2 and Table 2). In addition, axial diffusivity of IFOF.R ($\beta = -0.409$, $P = 0.005$ in SR 2) and ILF.R ($\beta = -0.512$, $P < 0.001$) in SR 3 was significantly and negatively correlated with attention performance (TMT-A Time) after Bonferroni correction (Fig. 2 and Table 2).

### Correlations Between WM Integrity and Gray Matter Density

Based these above results, we found that the IFOF.R and ILF.R are important fiber tracks with differences for patients with T2DM at different cognitive stages and that these regions are closely related to cognitive function. Therefore, we further explored the relationship between these two fiber tracks and the gray matter structures connected to them. The lingual.R and Frontal_Mid_Orb.R were anatomically connected to IFOF.R. Correlation analysis results showed the gray matter density of lingual.R was significantly related to the axial diffusion metrics of IFOF.R in SR 1 ($\beta = -0.519$, $P < 0.001$) and SR 2 ($\beta = -0.327$, $P = 0.004$) in T2DM patients (Fig. 2). The Temporal_Inf.R and Occipital_Mid.R were anatomically connected to the ILF.R. Correlation analysis results showed the gray matter density of right Temporal_Inf.R was significantly related to the axial diffusion metrics of ILF.R in SR 3 ($\beta = 0.376$, $P = 0.001$) and SR 4 (no significance) in T2DM patients (Fig. 2).

**Table 1—Demographic and neuropsychological test results**

| | T2DM-aMCI | T2DM-NC | HC | *F* value ($t/\chi^2$) | *P* value |
|---|---|---|---|---|---|
| | *n* = 36 | *n* = 40 | *n* = 40 | — | — |
| Sex, *n* | | | | 0.054 | 0.973# |
| Male | 13 | 15 | 14 | | |
| Female | 23 | 25 | 26 | | |
| Age (years) | 65.97 ± 7.81 | 65.35 ± 7.77 | 66.48 ± 7.46 | 0.215 | 0.807 |
| Education (years) | 10.89 ± 3.64 | 11.88 ± 3.16 | 12.46 ± 2.99 | 2.219 | 0.113 |
| Diabetes duration (years) | 11.13 ± 8.05 | 8.92 ± 6.29 | — | 1.19 | 0.238 |
| BMI (kg/m$^2$) | 27.46 ± 7.59 | 25.51 ± 3.33 | 24.03 ± 4.87 | 1.98 | 0.144 |
| Hypertension, *n* (%) | 25 (69) | 23 (58) | 16 (40) | 6.78 | 0.034#† |
| Hyperlipidemia, *n* (%) | 13 (9) | 22 (55) | 11 (27) | 6.59 | 0.037#‡ |
| Cerebrovascular disease, *n* (%) | 10 (28) | 7 (18) | 1 (3) | 9.42 | 0.009#† |
| General mental status | | | | | |
| MMSE | 25.26 ± 4.86 | 27.78 ± 1.56 | 29.43 ± 0.75 | 16.23 | <0.001†ξ |
| Episodic memory | | | | | |
| AVLT-Delay Recall | 1.18 ± 1.07 | 5.63 ± 2.64 | 8.55 ± 2.06 | 89.28 | <0.001†‡ξ |
| AVLT-Total | 14.49 ± 4.41 | 29.20 ± 9.62 | 40.38 ± 7.86 | 80.12 | <0.001†‡ξ |
| ROCF-Delay Recall | 8.9 ± 6.63 | 13.97 ± 7.15 | 20.48 ± 7.33 | 18.96 | <0.001†‡ξ |
| Working memory | | | | | |
| Digit Span | 10.97 ± 2.23 | 12.63 ± 2.27 | 13.18 ± 1.59 | 6.73 | 0.002†ξ |
| Digit Span Backward | 3.77 ± 1.18 | 4.95 ± 1.28 | 5.10 ± 0.96 | 8.42 | <0.001†ξ |
| Spatial processing | | | | | |
| ROCF-Copy | 31.61 ± 6.42 | 33.80 ± 2.94 | 34.08 ± 4.16 | 1.29 | 0.279 |
| Clock Drawing Test | 20.88 ± 5.56 | 24.97 ± 4.93 | 26.28 ± 3.30 | 12.22 | <0.001†ξ |
| Executive function | | | | | |
| Stroop Color and Word Test C-B time | 41.36 ± 16.83 | 40.25 ± 22.01 | 34.63 ± 22.13 | 1.29 | 0.279 |
| TMT-B time | 177.11 ± 52.11 | 163.35 ± 53.69 | 145 ± 49.29 | 3.73 | 0.028 |
| Language ability | | | | | |
| Boston Naming Test | 21.71 ± 4.47 | 24.40 ± 2.88 | 24.46 ± 2.94 | 5.08 | 0.008 |
| Category Verbal Fluency Test | 37.64 ± 9.69 | 44.85 ± 9.35 | 49 ± 7.07 | 8.62 | <0.001†ξ |
| Attention | | | | | |
| Symbol Digit Modalities Test | 28.96 ± 9.39 | 33.89 ± 9.44 | 38.55 ± 10.92 | 5.62 | 0.005† |
| TMT-A time | 84.63 ± 78.77 | 60.88 ± 17.69 | 53.15 ± 13.88 | 4.66 | 0.012 |
| Biochemical indicator | | | | | |
| $HbA_{1c}$ (%) | 6.49 ± 0.45 | 6.40 ± 1.31 | 4.93 ± 0.38 | 10.74 | <0.001†‡ |
| $HbA_{1c}$ (mmol/mol) | 47.37 ± 5.3 | 46.48 ± 4.29 | 30.31 ± 4.11 | 10.74 | <0.001†‡ |
| Total cholesterol (mmol/L) | 5.11 ± 0.73 | 4.80 ± 0.701 | 5.67 ± 1.10 | 0.904 | 0.427 |
| Triglycerides (mmol/L) | 2.13 ± 1.47 | 2.99 ± 1.99 | 1.55 ± 0.55 | 2.015 | 0.17 |
| HDL (mmol/L) | 1.29 ± 0.18 | 1.59 ± 0.29 | 1.38 ± 0.28 | 0.906 | 0.427 |
| LDL (mmol/L) | 3.01 ± 1.03 | 1.85 ± 0.09 | 3.52 ± 1.02 | 2.554 | 0.113 |

All subjects (T2DM-aMCI, T2DM-NC, HC) were matched for age, sex, and education. Values are the mean ± SD except where indicated. The comparisons of each cognition test among three groups were performed with ANCOVA. $P < 0.0036$ (0.05/14) was considered significant after Bonferroni correction. Post hoc pairwise comparisons were performed using *t* tests. $P < 0.017$ (0.05/3) was considered significant after Bonferroni correction. #The *P* value in the three groups was obtained using a $\chi^2$ test. Post hoc paired comparisons showed significant group differences between T2DM-aMCI and HC (†), between T2DM-NC and HC (‡), and between T2DM-aMCI and T2DM-NC (ξ).

## DISCUSSION

In the current study, several neuropsychological scores, including general mental status, episodic memory, working memory, spatial processing, language ability, and attention, exhibited intergroup gradual declines, with the best performance occurring in HC subjects, an intermediate performance in T2DM-NC patients, and the worst performance in T2DM-aMCI patients. Our subsequent analysis revealed that T2DM-aMCI patients had widespread WM disruptions and that T2DM-NC patients did not show any WM damage. In particular, the right IFOF and right ILF were significantly correlated with episodic memory and attention performance in T2DM patients. More importantly, cognitive impairment-related WM microstructural damage was associated with the degeneration of cortex connected to the affected WM tract.

This study examined DTI metrics to characterize comprehensive WM microstructural changes. FA measurements are the primary DTI-derived metrics believed to reflect the degree of alignment of cellular structures

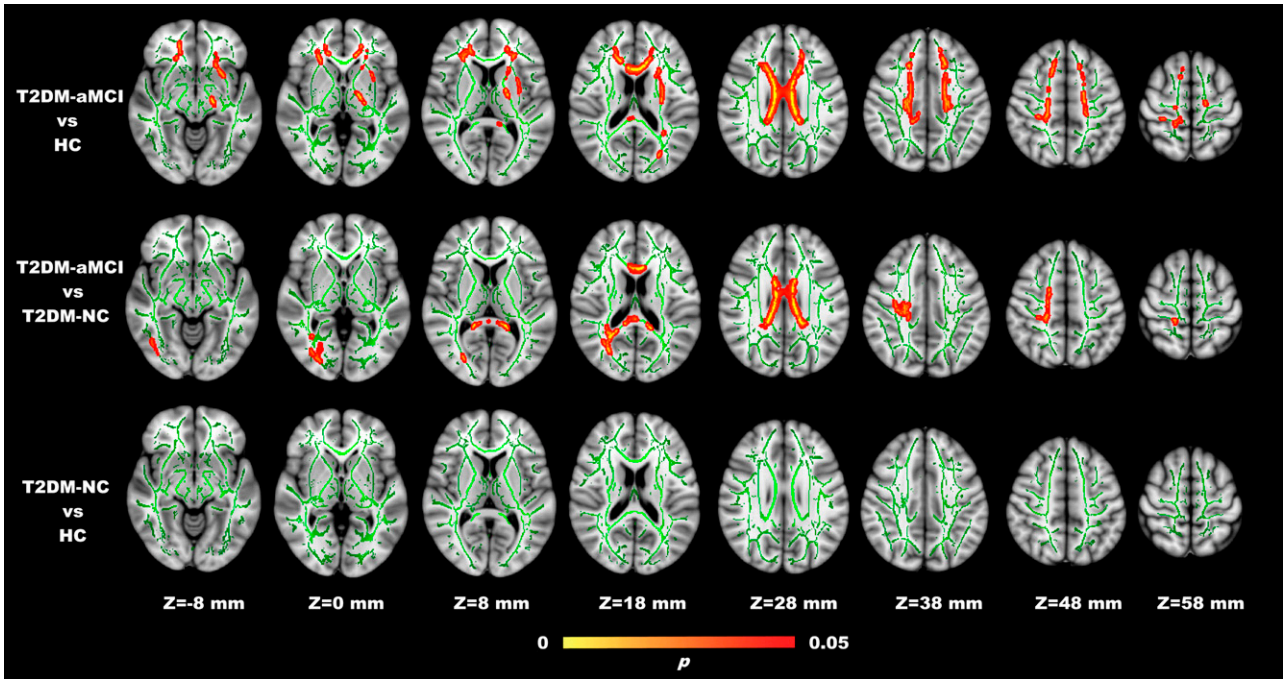


**Figure 1**—Voxel-wise Tract-Based Spatial Statistics differences in FA metrics between groups. Green represents the mean WM skeleton of all subjects. Top row: Red-yellow voxels (thickened for better visibility) represent the WM regions with decreased FA in the T2DM-aMCI patients compared with HC subjects (FWE corrected, $P < 0.05$). Middle row: Red-yellow voxels represent the WM regions with reduced FA in the T2DM-aMCI group compared with T2DM-NC group (FWE corrected, $P < 0.05$). Bottom row: There are no significant WM microstructural differences between the T2DM-NC patients and HC subjects.

within fiber tracts and their structural integrity (31). Although the specific cellular mechanisms underlying changes in FA measurements remain unclear in T2DM, our previous findings of decreased FA may be explained by the loss of axonal fibers, demyelination, or both (10).

Previous review studies have shown that T2DM and AD share several pathogeneses, including neurogenesis, blood-brain barrier, hyperglycemia, insulin resistance, and vascular dysfunction (32). Insulin resistance represents the main feature linking T2DM and the risk to develop AD and also seems to be an important determinant of axon pathologically related tau protein phosphorylation (33,34) and amyloid deposition (35), which are the critical pathology of AD. Given that insulin receptors are widely expressed and involved in the hippocampus and connected limbic brain structures, the most important brain region for learning and memory, it is plausible that decline of insulin signaling leads to cognitive impairment (36,37). A similar mechanism may also explain the observed significant relationships between axial diffusivity and cognitive performance in T2DM patients in Fig. 2. Accordingly, diffuse WM changes with a loss of myelin or axonal abnormalities have been well documented in almost all subtypes of T2DM and cognitive impairment patients (10,14,38), and T2DM-related cognitive impairment is related to axon pathologies. Moreover, previous study suggested that type 2 diabetes may be associated with aMCI through both AD and vascular pathology (2). We therefore speculated that the decreased WM integrity related to TMT-A in the results might be caused by vascular pathological factors of T2DM.

To explore the WM integrity change due to T2DM and T2DM-related cognitive impairment, we compared the difference of WM integrity between three groups. It was not surprising that the widespread decreased integrity in the cerebral WM skeleton in T2DM-aMCI patients compared with HC subjects was observed in the bilateral ATR and CST, right cingulate gyrus, and forceps major and minor, bilateral IFOF, and left UF. Especially, when the T2DM-aMCI group was compared with T2DM-NC patients without clinically confirmed cognitive impairment, the results were still considerably different in most regions, such as the right CST, forceps major and minor, right IFOF, right ILF, and right SLF. In addition, the T2DM-NC patients did not show significant reductions in WM integrity compared with the HC subjects. Thus, these results suggested that the observed changes in WM integrity are strongly coupled with cognitive impairment in T2DM patients with cognitive impairment. But previous studies suggest that T2DM-NC patients were observed to have microstructural WM abnormalities in multiple fibers such as the SLF, UF, ILF (12), and external capsule (39). The inconsistency of these results may be due to sample differences, with all of our patients from a community study and participants reported by Reijmer et al. (12) from a clinical study (part of the second Utrecht Diabetic Encephalopathy

**Table 2—WM integrity differences and its relationship with cognitive scores in all T2DM patients**

| | | Volumes ($mm^3$) | | Axial diffusivity and cognitive correlation | |
|---|---|---|---|---|---|
| Tracts | | T2DM-aMCI vs. HC | T2DM-aMCI vs. T2DM-NC | AVLT-Total | TMT-A time |
| 1 | ATR.L | 83 | 0 | — | — |
| 2 | ATR.R | 80 | 5 | — | — |
| 3 | CST.L | 102 | 0 | — | — |
| 4 | CST.R | 17 | 142 | — | — |
| 5 | CG.L | 28 | 0 | — | — |
| 6 | CG.R | 8 | 0 | — | — |
| 7 | CH.L | 0 | 0 | — | — |
| 8 | CH.R | 0 | 0 | — | — |
| 9 | Fmaj | 0 | 119 | 0.359** | — |
| 10 | Fmin | 369 | 13 | — | — |
| 11 | IFOF.L | 86 | 0 | — | — |
| 12 | IFOF.R | 111 | 63 | 0.441*** | −0.409** |
| 13 | ILF.L | 0 | 0 | — | — |
| 14 | ILF.R | 0 | 35 | 0.431*** | −0.512*** |
| 15 | SLF.L | 29 | 0 | — | — |
| 16 | SLF.R | 9 | 73 | — | — |
| 17 | UF.L | 66 | 0 | — | — |
| 18 | UF.R | 28 | 0 | — | — |
| 19 | SLF-temp.L | 8 | 0 | — | — |
| 20 | SLF-temp.R | 0 | 20 | — | — |

WM volumes were from the significant group differences (FWE corrected, $P < 0.05$). CG, cingulum (cingulate gyrus); CH, cingulum (hippocampus); Fmaj, forceps major; Fmin, forceps minor. **$P < 0.01$; ***$P < 0.001$.

Study). In addition, different methodologies—an ROI-based approach may help examine the overall change in fiber bundles, and voxel-based analysis may help identify areas of local variability on long fibers— may lead to different results.

More importantly, although previous studies have suggested that T2DM patients will have increases in microstructural injury in multiple WM fibers connecting distinct cortical and subcortical regions (10,12), the fibers that are related to T2DM-related cognitive disturbances are still unclear. Our findings clarify this relationship. The diffusion measures of the forceps major, IFOF, and ILF were correlated with cognitive test scores among T2DM patients, and these areas are involved performance of episodic memory and attention. It is possible that selective involvement of those tracts may represent the neural substrates that underlie the principal component of episodic memory and attention function due to the important role of these connecting tracts in cognitive functions in the brain. As supported by previous studies, the ILF, which is an occipitotemporal fiber bundle connecting occipital and temporal areas (40), was associated with attention-deficit disorder, slower processing speeds, and episodic memory performance in T2DM-NC patients and cognitively normal older adults (12,41,42). The forceps major, which connects the occipital lobes and crosses the midline via the splenium of the corpus callosum (43,44), is connected to the memory domain (45). The IFOF, which connects the frontal lobe with the posterior portions of the parietal and temporal lobes, mediates a direct communication between the occipital and frontal lobes (41,46) and is important in attention (47) as well as in episodic memory (48,49) in cognitive dysfunction in T2DM patients. In summary, T2DM damaged and accelerated patient cognitive ability mainly by attacking the ILF, IFOF, and forceps major. The aMCI-specific effects of T2DM suggest that T2DM had effects on the WM integrity primarily in patients with MCI and that the WM changes seen in the prodromal stages of dementia may be accelerated by T2DM.

Some limitations to our study should be noted. First, this is a cross-sectional study with a relatively small sample size; therefore, a longitudinal study with a larger sample size is needed in the future to achieve more convincing and accurate results. Second, participants with different levels of educational achievement may have shown different effects, although education was used as a control variable. Third, we only focused on the correlated relationship between the WM injury and gray matter atrophy in T2DM patients. Fourth, there was no aMCI-normoglycemic group. The causal relationship between these changes is still unclear. Fifth, it would be important to monitor the retinal disease information of patients to investigate the effect of retinal disease on the incidence of dementia. These factors should be included in future studies.

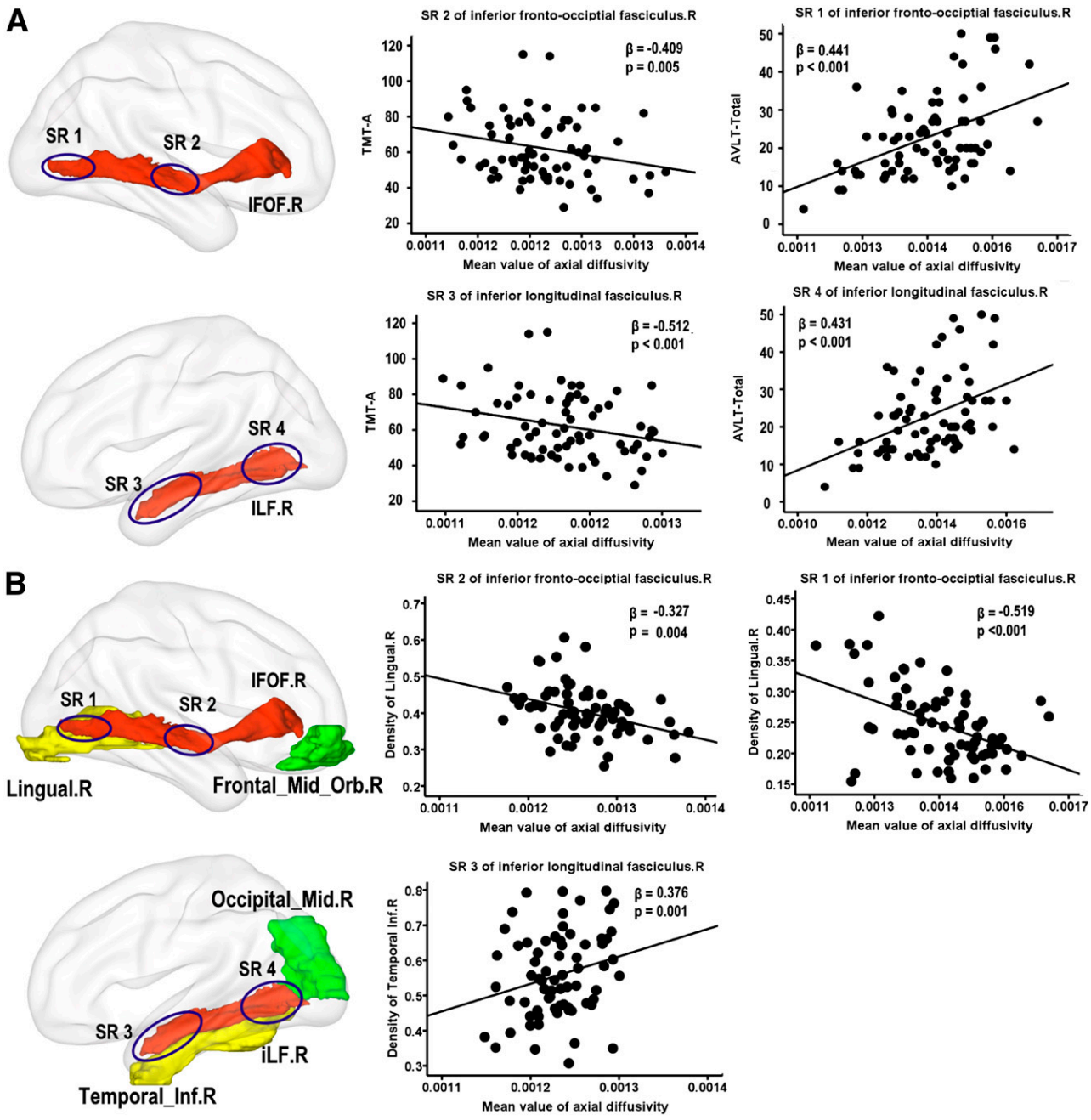


**Figure 2**—The relationship between WM integrity, cognitive scores, and gray matter density. *A*: The correlations between IFOF.R and ILF.R axial diffusivity and cognitive scores in T2DM patients. *B*: The correlations between IFOF.R and ILF.R axial diffusivity and gray matter density for areas anatomically connected to these two tracks. SR 1/4, the SR of AVLT-Total related to the axial diffusivity index; SR 2/3, the significant region of TMT-A related to the axial diffusivity index.

In summary, the current study revealed that T2DM-NC patients did not show any WM damage, and T2DM-aMCI patients showed significantly decreased WM integrity in widespread WM tracts, which suggests that significant WM changes occurred in cognitive impairment stages in T2DM patients. In particular, the episodic memory and attention function impairment in T2DM patients has significant correlations with the integrity decline of WM fibers in the right IFOF and right ILF. Moreover, WM microstructural damage related to cognitive impairment was associated with the degeneration of cortex connected to the affected WM tract.

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