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# Altered White Matter Integrity in the Corpus Callosum in Fibromyalgia Patients Identified by Tract-Based Spatial Statistical Analysis

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***Objective.*** **Although recent imaging studies of fibromyalgia (FM) have converged on a dysfunction of central pain processing as the primary pathophysiologic cause of the disorder, microstructural changes of the white matter (WM) suggestive of abnormalities in the anatomic connectivity of the brain have not been extensively examined. The aim of this study was to investigate WM integrity and its possible relationship to pain symptoms in women with FM.**

***Methods.*** **Nineteen FM patients and 21 age-, sex-, and education-matched healthy control subjects were included in the study and underwent diffusion-weighted imaging. Group differences in WM integrity, which were assessed via fractional anisotropy (FA), was investigated by applying tract-based spatial statistics.**

***Results.*** **As compared with the healthy control group, the FM group showed a single cluster with lower FA in the left body of the corpus callosum, which was found to be connected to the bilateral sensorimotor cortices ($P < 0.05$, corrected for multiple comparisons). Furthermore, FA values in the cluster were negatively associated with sensory pain, as measured by the Short-Form McGill Pain Questionnaire, as well as with the relative magnitude of sensory pain versus affective pain (calculated by dividing the sensory score by the affective score).**

***Conclusion.*** **Findings of the current study demonstrated that patients with FM had disrupted WM microstructure in the body of the corpus callosum, which was associated with clinical pain intensity. Our results suggest that abnormal interhemispheric transfer might contribute to the heightened pain perception. Our findings further strengthen the hypothesis of centrally augmented pain processing in patients with FM.**

Fibromyalgia (FM) is one of the most prevalent chronic pain disorders and is characterized by widespread chronic pain without evident damage to the soft tissues or muscles (1). Patients with FM usually have other clinical symptoms, such as fatigue, nonrestorative sleep, cognitive disruption, or episodes of depression (2).

Accumulating evidence from brain imaging studies supports the hypothesis that the most probable mechanism perpetuating FM is abnormal central pain processing (1). When either painful or nonpainful stimuli were delivered during functional magnetic resonance imaging (MRI) of the brain, FM patients exhibited widespread augmented activation in the somatosensory and insular cortices or reduced functional connectivity in the pain modulating network (3,4). In addition to the brain's response to experimentally induced pain, alterations of the resting-state functional connectivity (5,6), regional cerebral blood flow (7,8), and neurotransmitter

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concentrations (9,10) have been reported. These results suggest that FM patients have abnormal central pain processing that is closely related to enhanced nociception.

Dysfunctional central pain networks previously identified in functional imaging studies were confirmed by structural imaging studies in cortical and subcortical areas of the brain in FM patients. For example, the volume of gray matter (GM) or the thickness of the thalamus, hippocampus, amygdala, and the insular, somatosensory, prefrontal, and anterior cingulate cortex, except for the striatum, were reported to be decreased (6,11–15), indicating that prolonged nociception may have a negative effect on regional integrity in pain processing regions of the brain.

However, only a few studies have used diffusion tensor imaging (DTI) to investigate how white matter (WM) microstructures are altered in regions implicated in pain processing. Recent DTI studies of healthy subjects (16) and patients with chronic pain (17,18) have suggested that both fractional anisotropy (FA), which reflects WM integrity or the directionality of the molecular motion of water (19), and connectivity in the WM are sensitive in detecting chronic pain–related microstructural alterations and pain processing pathways.

Sundgren and colleagues (20) conducted the first DTI study and found reduced FA in the right thalamus. This reduction was also negatively correlated with clinical pain and an external locus of pain control. In a later study with a larger sample (30 FM patients and 30 healthy control subjects), Lutz et al (12) conducted a combined voxel-based morphometry/DTI study and reported reduced FA in the bilateral thalamic and insular regions and thalamocortical tract, as well as increased FA in the bilateral postcentral gyri, amygdalae, hippocampi, and superior frontal gyri, where GM volumes were found to be decreased. These structural abnormalities in the WM, but not the GM, were related to pain intensity, fatigue, and stress symptoms (12). However, a recent preliminary study of 10 FM patients and 10 healthy controls did not find any alterations in FA or in the apparent diffusion coefficient (21). These seemingly discrepant findings may be attributed to methodologic differences or insufficient sample size to yield a significant result.

This situation warrants further study, adopting a more sophisticated and objective approach to revealing the central mechanisms that contribute to chronic pain in FM. It should also be noted that the predefined and limited region-of-interest (ROI) method, which all previous DTI studies have used, deals only with a certain WM region and, thus, has the potential risk of missing subtle brain differences between study groups (22). Another important point to be stressed is that almost all of the existing structural neuroimaging studies have included patients with an advanced stage of FM, with a mean of >10 years since the onset of FM symptoms (6,12,13,15,23). This raises the need for research that develops a better understanding of the neural substrate of the early pathogenetic stage of FM and, thus, explores more effective therapeutic targets. We therefore planned to recruit patients who had experienced widespread pain linked to the diagnosis of FM for fewer than 10 years.

In this study, we aimed to investigate for the first time the structural integrity of the WM in patients with early-stage FM as compared with pain-free healthy controls through measurement of FA by applying tract-based spatial statistics (TBSS), which uses an FA skeleton, representing the center of the WM pathways across the study groups (24). TBSS has the advantages of circumventing not only the misalignment of FA images that can occur during their transport from the native space to the standard space, but also the rather arbitrary smoothing rate, both of which are inherent in previous voxel-based approaches (24). This technique is also investigator-independent. Thus, this method can provide increased objectivity when assessing regional differences in the WM. We further aimed to delineate the anatomic connectivity seeding from each region of the brain in FM patients with altered FA by applying probabilistic tractography. Last, we sought to determine whether FA was associated with the symptoms and duration of clinical pain.

We hypothesized that patients with FM would exhibit abnormalities in the integrity of fibers in the WM that are known to connect brain regions that have been implicated in abnormal pain processing. Specifically, we expected that patients with FM would show lower FA than healthy control subjects, since repetitive nociception and related stress would, in turn, lead to perturbation of the structural integrity (25).

## PATIENTS AND METHODS

**Study participants.** The study participants were recruited from university-based hospital rheumatology departments at Seoul National University Hospital and Hallym University Sacred Heart Hospital. All examinations, except for the diagnostic assessments and self-report questionnaires, were conducted at Seoul National University Hospital to ensure reliability of the results. Eligibility criteria for the FM patients were as follows: 1) meeting the American College of

Rheumatology 1990 criteria for primary FM (2), 2) having a duration of widespread pain of at least 3 months but <10 years, 3) reporting pain during the previous week of at least 40 on a 0–100-mm visual analog scale (VAS; where 0 = no pain and 100 = worst possible pain), 4) being female, 5) being between the ages of 30 and 60 years, and 6) being right-handed. Patients were excluded if they had secondary FM associated with inflammatory arthritis, a history of substance abuse, symptoms of peripheral neuropathy, or concomitant acute pain in the upper extremities or if they were pregnant or breastfeeding.

The study design included electrophysiologic assessments to be performed on the same day as the MRI acquisition. Thus, all potential participants were asked to stop any medications that might affect brain electrophysiology, such as analgesics, antidepressants, and anticonvulsants, 3 days before the start of the experiment. Those who agreed to stop medications were finally recruited for study.

Healthy control subjects matched to the FM patients according to age, sex, and handedness were recruited through local advertisements. They did not meet any criteria for chronic pain, nor did they meet criteria for a history of an axis I psychiatric illness (i.e., major depressive disorder, schizophrenia) or substance abuse.

Among the participants who met the above inclusion criteria, those with safety concerns for MRI acquisition (i.e., pacemaker, metal insert) were also screened and excluded. The study protocol was approved by the Institutional Review Boards at Seoul National University Hospital (H-1107-013-367) and Hallym University Sacred Heart Hospital (2011-I048) and was conducted in compliance with the Declaration of Helsinki. All participants (19 FM patients and 21 healthy controls) provided written informed consent prior to participation in the study.

**Image acquisition and analysis.** Diffusion-weighted scans were acquired on a research-dedicated 3T magnetic resonance whole-body scanner (Siemens Magnetom Trio Tim). Diffusion-weighted single-shot spin-echo echo-planar acquisition used the following parameters: repetition time (TR)/echo time (TE) 11,400/88 msec, flip angle 90°, bandwidth 1,698 Hz/pixel, matrix size 128 × 128 pixels, voxel size 1.9 × 1.9 × 3.0 mm, 45 contiguous axial slices of 3.0 mm in thickness, diffusion-sensitizing gradient directions 64, and a b factor of 1,000 seconds/mm$^2$, with no diffusion weighting (b0) image.

T1-weighted images were acquired to calculate global brain volumes, using the following parameters: sagittal acquisition with a 256 × 256–pixel matrix, field-of-view (FOV) 250 mm, slice thickness 1.0 mm with no gap, TR/TE 1,670/1.89 msec, flip angle 9°, and number of excitations (NEX) 1. To screen for mass lesions and pathologic hyperintensities of the brain, T2-weighted images were also acquired, using the following parameters: axial acquisition with a 640 × 580–pixel matrix, FOV 220 mm, slice thickness 5.0 mm, TR/TE 5,160/91 msec, flip angle 131°, and NEX 1.

A neuroradiologist who was blinded with regard to the diagnosis evaluated all of the images. All participants who were included in the study were free of gross abnormalities of the brain.

One investigator (DJK) visually checked all image volumes before preprocessing to screen noisy images that could bias a processing step as well as a result. We excluded subjects whose MRI examination could not be completed because of claustrophobia (n = 1) and those whose MR images had large signal variation or ghosting due to head motion (n = 1) or had hardware artifacts (n = 1). Thus, a total of 19 FM patients and 18 healthy control subjects were included in the following analyses.

Diffusion tensor analyses were performed using the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL) version 4.1 (Oxford University; www.fmrib.ox.ac.uk). The b0 image of each subject was extracted using the Brain Extraction Tool. Raw gradient volumes were motion artifact–corrected and eddy-current distortion–corrected by affine registration on a nongradient b0 volume. An FA map, which reflects the degree of directional strength within a voxel, was generated by fitting a diffusion tensor model to the corrected data using the dtifit function in the software. Axial diffusivity (AD), radial diffusivity (RD), and mean diffusivity (MD) maps were also generated in the same manner.

Next, TBSS processing was conducted to perform voxelwise statistical analysis at a predefined WM pathway (24). Briefly, each subject's FA map was aligned to a common template, a 1-mm isotropic FA image (FMRIB58_FA), using the Nonlinear Registration Tool in FMRIB. Warped images of all participants were averaged and thinned to create a mean FA skeleton, with an FA threshold of >0.20. Each subject's aligned FA map was projected onto the nearest relevant tract center of the mean FA skeleton by searching perpendicular to the local skeleton structure, which circumvents misalignment during registration. The mean FA values over the WM skeleton in each individual were calculated to assess the global difference between groups, which may potentially contribute to localized differences in FA. A nonparametric unpaired 2-sample *t*-test was performed using the "randomize" function, with 5,000 Monte Carlo permutation tests (26). To confirm possible effects of the mean FA of the whole skeleton or age on regional differences in FA, ancillary analyses were conducted that included those variables in the initial model. Significance was determined at a cluster size threshold of $t > 2.0$, corrected at $P < 0.05$ for multiple comparisons. We used the atlas tool (Harvard–Oxford cortical and subcortical structural atlases, Johns Hopkins University DTI-based WM atlases, and Jülich histologic atlas) implemented in FSL to determine the anatomic localization of the significant cluster.

To additionally provide information about potential microstructural changes related to FA changes, we assessed the AD (principal diffusion direction; $\lambda 1$), a measure of axonal integrity, the RD (perpendicular to the principal diffusion component; $[\lambda 2 + \lambda 3]/2$), the degree of myelination, and the MD (overall magnitude of water diffusion; $[\lambda 1 + \lambda 2 + \lambda 3]/3$), the magnitude of water diffusion (19,27), and compared them between the study groups. The cluster showing altered FA was binarized and then deprojected from the standard to the native space. The deprojected cluster for each subject was used as a mask in extracting the mean values for the AD, RD, and MD.

To further delineate an anatomically relevant tract of the regions of altered FA from the TBSS analysis in patients with FM, a seed-based probabilistic tractography analysis was performed. This technique provides a quantitative measure of the connection probability linking a seed region to each voxel

(28). Thus, we reasoned that this method might help in explaining the neural substrate of the disease.

The processing steps were as follows. The probability distribution function of the primary diffusion direction over the whole brain was calculated using the BedpostX algorithm (29). The seed cluster derived from the TBSS analysis in standard space was back-projected by applying an inverse-warping matrix generated at the TBSS normalization step. Probabilistic tractography consisted of 5,000 individual streamlines from every voxel within the seed cluster to every other voxel, based on the probability distribution function, with a maximum step number of 2,000 and a step length of 0.5 mm. This was conducted using the Probtrackx algorithm on the individual diffusion space. Tracking was terminated with a curvature threshold of 0.2 (corresponding to 80°). The individual tractogram was normalized by dividing by the "waytotal" to control for interindividual variability in the brain and tract size, and was thresholded at 1% to leave the tract with a high likelihood of being connected to the seed cluster. Thresholded and binarized tracts in the native space were transformed to the standard space by applying a warping matrix derived from the TBSS nonlinear registration. The mean group-specific map for each FM patient and healthy control subject was generated by averaging the individual tractograms in the standard space and was thresholded at 0.3 for visualization.

**Clinical scales and questionnaires.** Handedness was classified according to the Edinburgh Handedness Inventory (30). The severity of depression and anxiety symptoms was measured by the Beck Depression Inventory (BDI) (31) and the Beck Anxiety Inventory (BAI) (32), respectively. Subjective sleep quality was assessed with the Pittsburgh Sleep Quality Index (33). The Fibromyalgia Impact Questionnaire (FIQ) (34) was used to assess the composite impact of FM symptoms, including pain, stiffness, fatigue, sleep disturbances, and psychological stress. Each sensory and affective component of pain quality was assessed by the Short-Form McGill Pain Questionnaire (SF-MPQ) (35). The study consisted of 2 sessions conducted on 2 different days (mean ± SD interval between sessions 8.1 ± 6.6 days). Clinical outcome was assessed at the first visit, and DTI data were acquired at the second visit.

**Statistical analysis.** Continuous variables of demographic, clinical symptom severity, and whole-brain measures were tested by independent 2-sample *t*-test, and categorical variables were tested by chi-square test.

FA values at local maxima within each significant cluster were extracted to test the relationship to clinical variables, including pain duration, pain during the previous week (by VAS), SF-MPQ subscale scores, and FIQ scores, by using Pearson's correlation coefficient. *P* values (2-tailed) less than 0.05 were considered significant. All continuous variables were assessed for normal distribution by Kolmogorov-Smirnov test.

## RESULTS

**Characteristics of the study participants.** Demographic and clinical characteristics of the study participants are shown in Table 1. The FM group did not differ significantly from the healthy control group in terms of age, years of education, or handedness. The mean duration of widespread pain was 35.6 months (range 3–115 months). Scores on the BDI and BAI in the FM group indicated that the patients had mild-to-moderate depression and moderate-to-severe anxiety at the time of assessment. In addition, patients with FM reported poorer sleep quality compared with the healthy controls.

**Whole-brain measures.** We calculated the mean FA from the whole FA skeleton for each study group. These values were compared by *t*-test for independent samples. Although the FM group exhibited lower mean FA (mean ± SD 0.456 ± 0.01) than the healthy control group (0.461 ± 0.01), these differences did not reach statistical significance ($t = 0.98$, $P = 0.33$). To provide a landscape view of the whole-brain morphology, T1-weighted images in the native space were spatially normalized and segmented into GM, WM, and cerebrospinal fluid (CSF) using a unified segmentation algorithm implemented in Statistical Parametric Mapping 5 (Wellcome Trust Centre for Neuroimaging), running on Matlab 7.9.0 (MathWorks) (36). Each GM, WM, and CSF map was then modulated with deformation fields to adjust for volume changes during the normalization step. Last, the GM, WM, and CSF volumes and the total intracranial volume (sum of the GM, WM, and CSF volumes) were calculated using custom Matlab code (http://www.cs.ucl.ac.uk/staff/G.Ridgway/vbm/get_totals.m). The results demonstrated that the intergroup differences in whole-brain volume measures were not statistically significant (Table 1).

**Integrity of the lower corpus callosum in FM patients.** Compared with the healthy control group, the FM group exhibited a single cluster with lower FA in the left body of the corpus callosum lateral to the cingulum and anterior cingulate cortex (452 voxels within the significant cluster). A local maximum standard was located at the Montreal Neurological Institute (MNI), showing x,y,z coordinates of −14,0,33 within the body of the corpus callosum. This cluster was enlarged for visualization purposes using the tbss_fill function in FSL (Figure 1).

Ancillary analyses in which each potential covariate (mean FA of the whole FA skeleton or age) were included in the model led to similar results. There were no regions where FA values were greater in the patient group than in the healthy control group.

**Findings of the within-cluster analyses.** We additionally tested intergroup differences in AD, RD, and MD in the cluster of subjects with lower FA by *t*-test for

**Table 1.** Demographic and clinical characteristics of the study participants*

| | Fibromyalgia patients (n = 19) | Healthy controls (n = 18) | *t* statistic, 35df | *P* |
|---|---|---|---|---|
| Demographic features | | | | |
| Age, years | 44.9 ± 8.3 | 44.7 ± 8.8 | 0.08 | 0.94 |
| Education, years | 13.1 ± 2.2 | 12.9 ± 2.9 | 0.13 | 0.90 |
| Edinburgh score | 83.0 ± 19.8 | 88.6 ± 13.4 | 1.00 | 0.32 |
| Clinical features | | | | |
| Age at onset, years | 42.9 ± 8.5 | NA | NA | NA |
| Duration of pain, months, no. (%) of patients | 35.6 ± 31.1 | NA | NA | NA |
| ≤12 months | 5 (26.3) | NA | NA | NA |
| 13–36 months | 8 (42.1) | NA | NA | NA |
| 37–60 months | 2 (10.5) | NA | NA | NA |
| 61–120 months | 4 (21.1) | NA | NA | NA |
| Beck Depression Inventory score | 19.0 ± 6.8 | 3.2 ± 4.1 | 8.44 | <0.001 |
| Beck Anxiety Inventory score | 23.3 ± 10.8 | 1.8 ± 2.2 | 8.25 | <0.001 |
| Pittsburg Sleep Quality Index score | 13.0 ± 3.4 | 3.3 ± 1.2 | 11.44 | <0.001 |
| Pain symptom severity | | | | |
| Past week pain, by VAS, mm | 51.8 ± 19.3 | NA | NA | NA |
| FIQ score | 62.5 ± 13.2 | NA | NA | NA |
| SF-MPQ score | | | | |
| Sensory | 14.7 ± 6.8 | NA | NA | NA |
| Affective | 5.8 ± 2.6 | NA | NA | NA |
| Total | 20.5 ± 8.8 | NA | NA | NA |
| Global brain volume, $cm^3$ | | | | |
| Gray matter | 643.6 ± 49.2 | 665.2 ± 58.6 | 1.22 | 0.23 |
| White matter | 457.8 ± 39.5 | 469.2 ± 44.4 | 0.83 | 0.41 |
| Cerebrospinal fluid | 459.3 ± 88.1 | 430.1 ± 93.8 | 0.98 | 0.33 |
| Total intracranial volume | 1,560.7 ± 135.0 | 1,564.5 ± 119.8 | 0.09 | 0.93 |

* Of the 40 study participants, only the 37 whose imaging data were analyzed are represented here. The visual analog scale (VAS; 0–100 mm) score for pain during the previous week was obtained immediately before magnetic resonance imaging was performed. Except where indicated otherwise, values are the mean ± SD. NA = not applicable; FIQ = Fibromyalgia Impact Questionnaire; SF-MPQ = Short-Form McGill Pain Questionnaire.

independent samples. RD was found to be greater in the FM patients than in healthy controls ($t = 3.06$, $P = 0.004$). The FM group had lower AD compared with the healthy control group only at a trend level of significance ($t = 1.95$, $P = 0.060$). There was no corresponding intergroup difference in MD ($t = 1.27$, $P = 0.20$) (Figure 2).

**Results of probabilistic tractography.** We further sought to derive structural connectivity seeding from the area in the body of corpus callosum where the FM

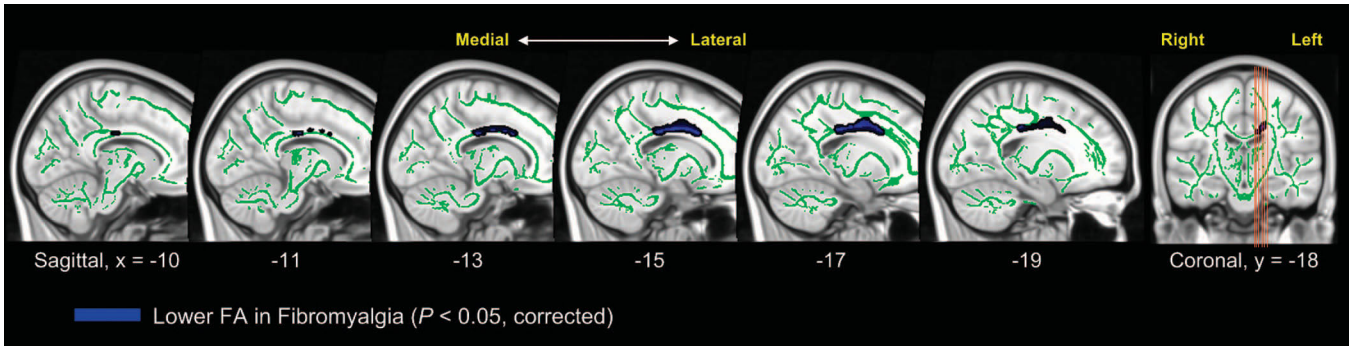


**Figure 1.** Lower fractional anisotropy (FA) in patients with fibromyalgia compared with healthy controls. Blue areas indicate regions of lower FA. For visualization purposes, regional differences on the FA skeleton were thickened in order to more easily find the result on the white matter skeleton (green). The red vertical lines in the image at the far right correspond to each of the sagittal slices. The local maximum standard from the Montreal Neurological Institute indicates the x and y coordinates within the body of the corpus callosum. The *P* value for the intergroup difference was determined by nonparametric unpaired 2-sample *t*-test.

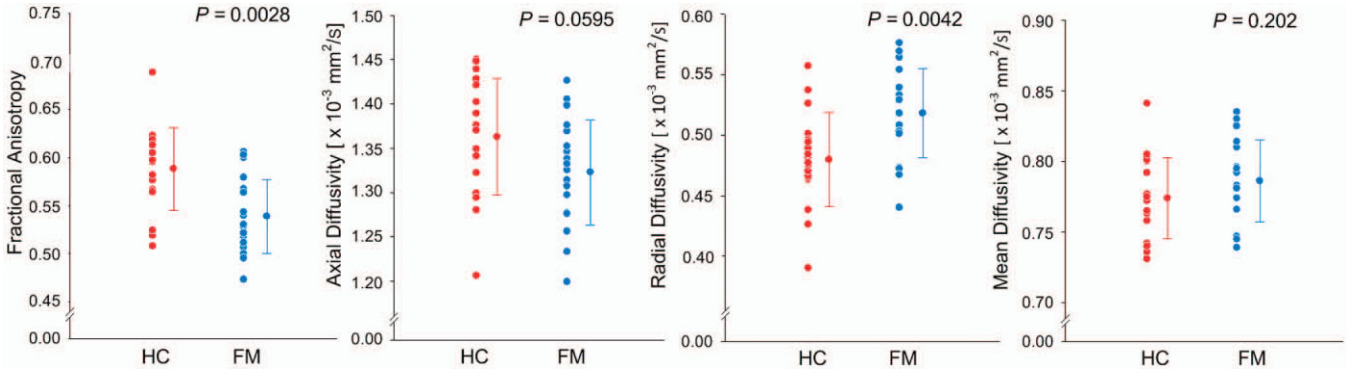


**Figure 2.** Diffusion parameters in the cluster of lower fractional anisotropy (FA) in fibromyalgia (FM) patients as compared with healthy control (HC) subjects. Scatterplots show the FA, axial diffusivity, radial diffusivity, and mean diffusivity values in the cluster showing lower FA in the patient group obtained in the individual native space. Bars show the mean ± SD. *P* values were determined by *t*-test for independent samples.

patients had lower FA than the healthy controls to any other voxel in the brain by using probabilistic tractography. We found that the callosum cluster was connected to the bilateral superior frontal, primary, and premotor cortices and the primary somatosensory cortices implicated in the pain processing pathway (Figure 3). The FM patient group and the healthy control group showed similar connectivity profiles.

**Correlation with symptom severity.** We found that the sensory pain score on the SF-MPQ was negatively associated with the FA at local maxima within the significant cluster identified by TBSS analysis (r =

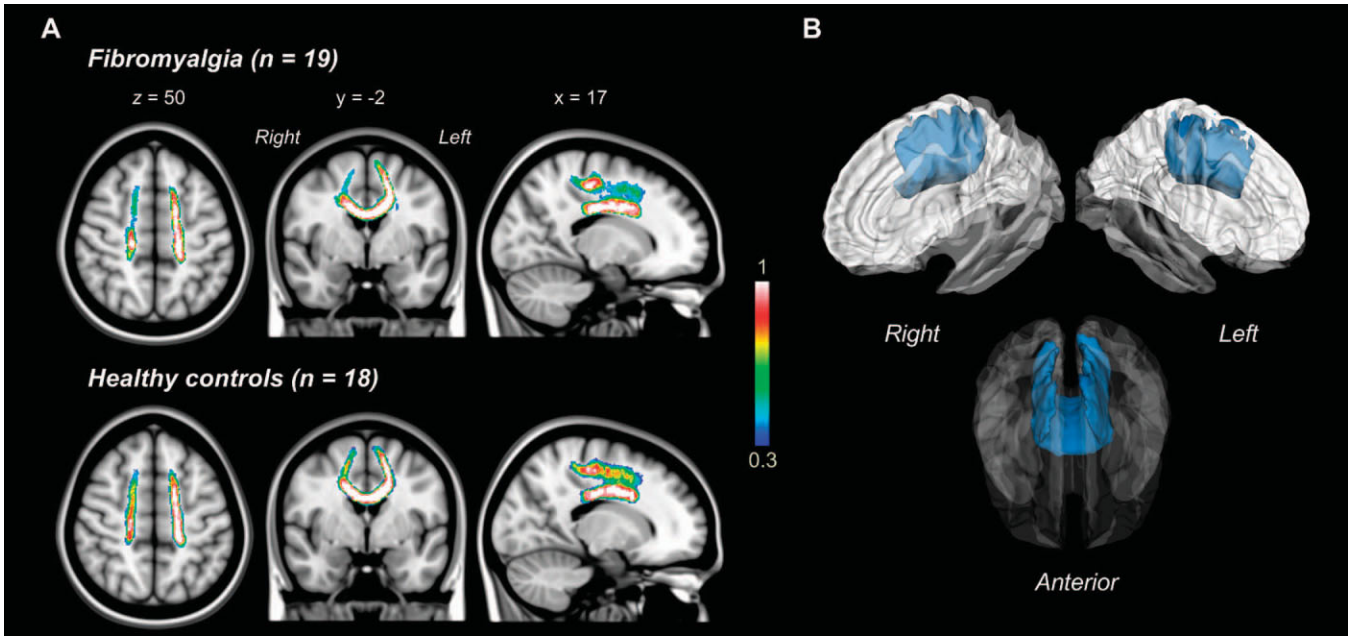


**Figure 3.** Connectivity of the corpus callosum. **A,** Results of probabilistic tractography starting from the cluster of decreased regional fractional anisotropy (the body of the corpus callosum) in the fibromyalgia (FM) patients. The reconstructed fiber tracts of each group were averaged and visualized on the Montreal Neurological Institute 152 T1-weighted standard brain. The color scale for each voxel represents a proportion of at least 30% of subjects in whom this voxel was connected by the seed cluster. **B,** Connectivity of the corpus callosum cluster to other regions of the brain. To provide information about the anatomic location of the nerve fiber tracts, the tracts identified larger than the proportion of 30% for only healthy controls were rendered into the 3-dimensional surface.

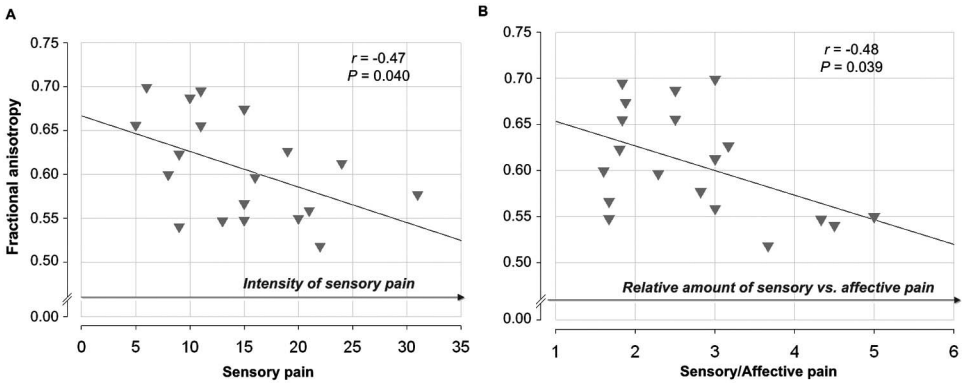


**Figure 4.** Correlation between sensory pain intensity and fractional anisotropy (FA) at the local maxima in the significant cluster identified on the tract-based spatial statistics analysis. **A,** FA values extracted at local maxima from clusters showing group differences correlated negatively with the sensory pain scores measured with the Short-Form McGill Pain Questionnaire. **B,** FA values also correlated negatively with the relative contribution of sensory pain scores versus affective pain scores.

$-0.47$, $P = 0.040$) (Figure 4A). We further tested whether sensory pain intensity after controlling the affective component (dividing the sensory pain score by the affective pain score on the SF-MPQ) was associated with FA, which was motivated by the analysis of the relative contribution of affective or sensory pain to $\mu$-opioid receptor availability (37). Interestingly, we also found a significant relationship between the relative amount of sensory pain and the FA values ($r = -0.48$, $P = 0.039$) (Figure 4B). However, the affective pain score was not associated with FA ($r = -0.20$, $P = 0.41$). In addition, scores on the VAS for pain in the previous week ($r = 0.09$, $P = 0.72$), the FIQ ($r = -0.01$, $P = 0.96$), and pain duration ($r = 0.31$, $P = 0.20$) were not associated with FA in the same location.

## DISCUSSION

The main goal of this study was to investigate possible microstructural alterations in WM tracts and their relationship to pain symptom severity in patients with FM. We found that patients with FM had lower FA in the left body of the corpus callosum in proximity to the cingulum bundle. The within-cluster analysis revealed that patients with FM had significantly greater RD and a trend toward lower AD in the corpus callosum as compared with healthy controls, suggesting demyelination or axonal damage to the corpus callosum fibers in the FM patients. In addition, correlational analyses revealed that FA in the corpus callosum cluster was negatively associated with sensory pain intensity.

Despite adopting a whole-brain approach to the identification of WM abnormalities in an unrestricted space, we did not observe any alterations in the WM FA in regions adjacent to the thalamus, insular cortices, and superior frontal cortices, unlike what we had assumed based on the findings of previous studies. We thought that the discrepant findings across studies might have resulted from differences in the clinical characteristics of the patients, including the sex ratio and disease duration or the analytical approaches and sample sizes. In terms of the disease duration, our FM group had a rather shorter duration since the onset of widespread pain (on average, 3 years) than the FM groups in previous DTI studies (on average, >10 years in the Lutz et al study [12]). Thus, this result might reflect a rather early stage of WM characteristics in FM patients.

Our study is the first to use TBSS analysis to identify lower FA in the body of the corpus callosum in patients with relatively early-stage FM. This finding is interesting, since previous DTI studies expected to observe an abnormality of FA within the splenium of the corpus callosum (50-$mm^3$ ROI) and did not derive a significant result in the splenium (20,21). Considering the connection of the corpus callosum fibers to the cortices (38), we reasoned that the pathophysiology of FM would be better reflected in the body of the corpus callosum than in the splenium.

In analyses aimed at explaining the underlying biologic implications of these FA changes, we observed significantly greater RD and a trend toward lower AD in an area of the brain where lower FA was observed in the patients with fibromyalgia. Fractional anisotropic values were reported to be highly correlated with the axonal membrane circumference and packing density (27). In

an experimental mouse brain, demyelination increased RD, but not AD, in the corpus callosum (27,39). It has also been reported that the degree of myelination can modulate FA in certain ROIs (40). We reasoned that our finding of lower FA, together with previous experimental data, may reflect axon damage, demyelination, or dysmyelination in that area. It should be noted that measures of FA, AD, RD, and MD are complementary measures that are sensitive to microstructural changes. However, it cannot directly indicate a specific change within the WM. In addition, lower FA might be induced by macrostructural differences between the 2 samples, such as the number of crossing fibers in a voxel (40).

Analyses using probabilistic tractography revealed that pathways seeding from the corpus callosum cluster were identified as transcallosal fibers connecting bilateral sensorimotor and superior frontal cortices, consistent with extensive research in corpus callosum tracts using tractography (38). The corpus callosum is the largest WM tract in the brain and plays a crucial role in interhemispheric communications by physically connecting homologous cortical regions of each hemisphere. Together with the frontal cortices, the corpus callosum has been suggested to influence the effectiveness of the frontal cortices in gating or filtering sensory inputs (41). This argument has been supported by the findings that individuals with higher hypnotizability (exhibiting attentional and inhibitory capabilities in hypnotic analgesia) had a larger rostrum of the corpus callosum than did individuals with lower hypnotizability (42).

There is evidence that connectivity of the callosum fibers would relate to interhemispheric inhibition. Wahl and colleagues (43) demonstrated that WM integrity of the corpus callosum was positively associated with transcallosally mediated interhemispheric inhibition between the motor cortices of both hemispheres, as assessed by paired-pulse transcranial magnetic stimulation. Based on these findings, we cautiously speculated that perturbation in mutual inhibitory influences between homologous hemispheres might enhance bilateral sensorimotor activity, which previous functional studies have suggested to be responsible for augmented pain in FM (3,44,45).

Also notable is that the WM integrity of the body of corpus callosum was positively associated with the capacity of working memory in healthy individuals (46). In addition, in the study correlating the working memory performance and the GM/WM volume in FM patients, verbal working memory was positively correlated with WM volume of the midcingulm/cingulate extending to the body of the corpus callosum (23). Although we did not assess the memory function of the study subjects, it is possible that reduced FA in the body of the corpus callosum may be related to the impaired working memory function in FM.

Interestingly, altered FA in the body of the corpus callosum, the most prominent finding of our study, has been observed in patients with other types of chronic pain, such as complex regional pain syndrome, temporomandibular joint disorder, and migraine (17,18,47). These findings of altered corpus callosum integrity may originate from the high comorbidity found across these disorders (48) and/or be indicative of corpus callosum dysfunction in chronic pain, considering the suggested role of the corpus callosum in inhibitory control of pain (42).

Most previous functional imaging studies considered each brain region of pain processing in isolation. However, our study result suggests that the corpus callosum–mediated interhemispheric connection may contribute to clinical sensory pain. This argument is supported by altered FA in the corpus callosum, as seen in the TBSS analysis, and is further supported by the negative correlation between the FA at local maxima and sensory pain as well as the relative amount of sensory versus affective pain. The SF-MPQ, especially the sensory pain score, is known to be sensitive in reflecting individual differences regarding how pain is perceived qualitatively in the resting state (35). Therefore, this relationship suggests that the integrity of the corpus callosum may mediate individual variations in clinical pain in patients with FM.

In interpreting the current findings, the following study limitations need to be acknowledged. First, this study is a cross-sectional design, which hinders any analysis of sequential causal relationships between microstructural abnormalities and widespread pain and vice versa. It is assumed that WM changes would result from repetitive nociceptive inputs via the mechanism of maladaptive plasticity, based on the finding that the duration of the FM considerably overrides the influence of aging on GM volumes (11). Second, WM changes might be affected by analgesic or antidepressant medication known to exert neuroplastic changes in brain structure and function (49). This study design could not rule out these effects. Third, the sample size of the study was modest. This may increase the risk of Type I error when comparing patients and healthy individuals and warrants a replication with larger samples. Future studies using a longitudinal neuroimaging design with larger samples could resolve these issues efficiently. Last, since

we studied only women with FM, our findings are not generalizable to men, especially considering the distinct differences of FA of the corpus callosum subregions between the sexes (50). Nonetheless, since women constitute the majority of the FM population, this study result can provide a meaningful insight into our understanding of the neural pathophysiology of FM.

In summary, the current study demonstrated that patients with FM had disrupted WM microstructure in the body of the corpus callosum associated with clinical pain intensity. Our results suggest that abnormal interhemispheric transfer might contribute to heightened pain perception and further strengthen the hypothesis of centrally augmented pain processing in FM.

## AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Chung had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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