# Microstructural Evidence of Neuroinflammation for Psychological Symptoms and Pain in Patients with Fibromyalgia

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**Abstract**

**Objectives** In patients with fibromyalgia (FM), the brain shows altered structure and functional connectivity, but the mechanisms underlying these changes remain unclear. This study investigated the associated changes in brain microstructures and neuroinflammation of patients with FM.

**Methods** We recruited 14 patients with FM and 14 healthy controls. Visual analog scale (VAS), Beck's Anxiety Inventory (BAI), and Beck's Depression Inventory-II (BDI-II) were used for assessing their pain, anxiety, and depression levels, respectively. Diffusion kurtosis imaging (DKI) was used to visualize microstructural alterations associated with neuroinflammation in specific brain regions. The biomarkers for the neuron damage, including serum tau and amyloid β protein fragment 1-42 (Aβ1-42) levels, were assessed. Spearman correlation of DKI parameters with VAS, BAI, and BDI-II scores and tau and Aβ1-42 levels were assessed.

**Results** The patients with FM had significantly higher levels of Aβ1-42 levels compared with the controls. Compared with the controls, the patients showed significantly lower DKI parameters in the bilateral dorsal–lateral prefrontal cortex and orbital–frontal cortex. The patients showed a significant correlation between the axial kurtosis values of the amygdala and VAS scores (left: rho = −0.603, $p$ = 0.022; right: rho = −7.04, $p$ = 0.005).

**Conclusions** To the best of our knowledge, this is the first study to use DKI to examine the brain of FM patients. We noted significant DKI changes at specific areas associated with neuroinflammation in patients with FM. Our results provide valuable information on brain neuroinflammation and pathophysiological changes in patients with FM.

**Keywords**: fibromyalgia; diffusion kurtosis imaging; neuroinflammation; pain; depression; anxiety

## key messages

1. Using DKI showed microstructural evidence of inflammation in the DLPFC and OFC of FM patients.
2. Altered DKI parameters of the amygdala was correlated with VAS of pain in FM patients
3. We found negative correlations between mean Aβ1-42 and DKI in the DLPFC of FM patients.

## Introduction

Fibromyalgia (FM) is a relatively common chronic pain disorder. The pathomechanism of FM may involve pain dysregulation in the central nervous system (CNS)(1). The pain transmission and modulation pathways involving specific areas such as the mesolimbic system, anterior cingulate cortex (ACC), and insula are altered in patients with FM(2). Functional magnetic resonance imaging (fMRI) highlighted the presence of abnormal pain and other sensory processing in patients with FM(3). Furthermore, patients with FM exhibit structural changes in the brain, such as regional grey and white matter loss(4). Diffuse tensor imaging (DTI), which can provide information on brain microstructure integrity, is used to evaluate neural tracts(5). Significant decreases in fractional anisotropy in the bilateral thalami, the thalamocortical tracts, and insular regions have been reported in patients with FM. In these patients, pain intensity scores were correlated with DTI parameters in the right superior frontal gyrus(4). The altered regional brain microstructure is associated with symptom profiles in patients with FM.

The underlying mechanisms of structural changes in patients with FM remain unclear. Accumulating evidence shows that abnormal glial cell activation and neuroinflammation are key factors associated with chronic pain(6). Substance P, glutamate, nerve growth factor, and brain-derived neurotrophic factor levels are higher

in the cerebrospinal fluid of patients with FM(7). These substances can activate microglia and astrocytes, leading to the release of proinflammatory cytokines such as interleukin (IL) 1β and IL-8. IL-8 is positively associated with increased pain intensity in patients with FM(7).

Diffusion kurtosis imaging (DKI) estimates the kurtosis of water diffusion probability distribution function. The orientation of water diffusion is affected by changes in cellular membranes, intracellular organelles, and myelinated axons in the brain(8). The DKI method can provide detailed information about microstructural and microenvironmental changes in the brain(9). Compared with DTI, DKI can more accurately detect microstructural alterations in the brain of patients with major depression(10). A mouse model study showing neuroinflammation was associated with a significant decrease in regional DKI parameters such as mean kurtosis (MK), radial kurtosis (RK), and axial kurtosis (AK)(11). An autoimmune encephalomyelitis study used DKI parameters to investigate neural damage as indicated by inflammatory lesions, demyelination, and axonal damage and supported using DKI-derived biomarkers to detect neuroinflammation(12).

No data regarding the DKI features in the brain of patients with FM are available. In the present study, we used DKI to investigate the microstructural changes for detecting focal neuroinflammation of the brain in patients with FM. Further, we

examined the correlations between DKI parameters, blood biomarkers of neurodegeneration and neuroinflammation, psychological symptoms, and pain in FM patients. Our results can provide valuable information on the roles of neuroinflammation in patients with FM.

## Methods

### Participants

This study included 14 female patients with FM (mean age: 50.1 years old) and 14 healthy female controls (mean age: 56.1 years old). Each participant provided her informed consent. The Joint Institutional Review Board approved this study of the Taipei Medical University (N201812078). The FM group consisted of patients who met the American Congress of Rheumatology 2016 criteria for FM from Taipei Medical University Hospital. We excluded patients who (1) had a malignancy; (2) had an active or chronic infection; (3) had dementia, a CNS disorder, or head trauma; (4) had an endocrine disorder; (5) had a major autoimmune disorder such as systemic lupus erythematosus and rheumatoid arthritis, and (6) were pregnant. The control group comprised healthy people without a current history of known disease and drug use. The patients were requested to stop taking medications, except acetaminophen, for at least two weeks before clinical evaluation and blood analysis. For blood analysis, 10 mL of blood was drawn from a forearm vein after the participants woke up in the morning but before they had any meals.

### Questionnaires

Beck's Depression Inventory-II (BDI-II) and Beck's Anxiety Inventory (BAI) are 21-item self-reported questionnaires to assess depression and anxiety severity. Each

item is rated from 0 (not severe at all) to 3 (very severe), with a maximum possible score of 63. The visual analog scale (VAS) of pain is used to evaluate the average pain level. This scale is a 10-cm ruler, with markings ranging from 0 (no pain) to 10 (the worst imaginable pain). We requested that patients mark points corresponding to the average pain level in the past week.

**Immunomagnetic reduction assay (IMR) for tau protein and amyloid β protein fragment 1-42 (Aβ1-42)**

We used an IMR to measure blood tau protein (tau) and Aβ1-42 levels. IMR uses antibody-functionalized magnetic nanoparticles. The concentrations of detected molecules are calculated by the magnetic susceptibility associated with the interaction between the magnetic nanoparticles and molecules. A superconducting-quantum-interference-device achieves ultra-high sensitivity for detecting extremely low concentrations of Aβ1–42 and Tau. The study has revealed the high consistency in IMR analysis. IMR can precisely assay the Aβ1–42 or Tau-protein concentrations at several tens of pg/mL compared with ELISA(13). The magnetic reagents MF-AB2-0060 and MF-TAU-0060 were used to assay the serum biomarkers Aβ1-42 and tau on the Xacpro-S detector (MagQu, New Taipei City, Taiwan).

**MRI data acquisition and DKI data analysis**

All brain images were acquired on a 3T MRI system (MAGNETOM Prisma; Siemens, Erlangen, Germany) equipped with a 20-channel head coil. To obtain an anatomical reference, we performed high-resolution T1-weighted imaging using a 3D magnetization-prepared rapid gradient echo sequence: repetition time (TR)/echo time (TE) 2,000 ms/2.3 ms, flip angle 8°, field of view (FOV) 240 × 240 mm, acquisition matrix 256 × 256, all of which resulted in an isotropic spatial resolution of 1 $mm^3$A whole-brain diffusion-weighted sequence was applied with the following parameters: TR/TE 5,700 ms/84 ms, slice thickness 2.70 mm, and acquisition matrix 82 × 82. Diffusion weighting with two b factors 1,000 and 2,000 s/$mm^2$ was performed along 64 directions, complemented by one scan without diffusion gradient.

FMRIB Software Library (FSL, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki) was used for the pre-processing of the MRI images, included skull stripping, motion correction, and DKI was registered to the first acquired b0 image on all diffusion-weighted images for motion correction. Local principal component analysis and correction were conducted before image reconstruction. Targeted ROIs were selected by using the talairach space. and the pre-processed b0 image was registered with the standard space by FSL *flirt* command in the affine transformation method. DKI data were analyzed using an in-house program with MATLAB (MATLAB 2021a, The MathWorks, Inc., Natick, US). We calculated three DKI parameters, including MK, AK, and RK in each targeted ROI

that contribute to the psychological domain of pain, including the insula, amygdala, hippocampus, dorsal–lateral prefrontal cortex (DLPFC), orbitofrontal cortex (OFC), and thalamus (Figure. 1A).

**Statistical analysis**

DKI parameters were analyzed using the independent two-tailed *t* test to compare the differences between the FM and control groups. Spearman's rho was calculated to investigate the correlation of DKI parameters with Aβ1-42 levels and anxiety scores. The significance level was set to at $p < 0.05$. We applied the Bonferroni correction for correcting multiple comparisons with the false discovery rate (FDR) set at $p < 0.05$ level. We used SPSS (version 19.0; SPSS, Chicago, IL, USA) to perform data analyses.

**Results**

The patients with FM had significantly higher scores on BAI (13.4 ± 8.9 vs. 4.2 ± 3.6, $p$ = 0.001) and BDI-II (11.4 ± 8.6 vs. 3.4 ± 5.9, $p$ = 0.003) compared with the controls. Tau levels showed no significant difference between the two groups. Aβ1-42 levels were significantly higher in the patients (17.05 ± 0.45 pg/mL) than in the controls (16.52 ± 0.39 pg/mL, $p$ = 0.003; Table 1). MK and RK values in the bilateral DLPFC and bilateral OFC and the AK value in the left DLPFC were significantly lower in the patients with FM than in the controls (Fig. 1C). Spearman's correlations of DKI parameters with mean Aβ1-42 levels and BAI and BDI-II scores with the Bonferroni correction for correcting multiple comparisons with the FDR were examined. The results indicated negative correlations between mean Aβ1-42 and MK, AK, and RK in the left DLPFC of the patients with FM. The mean Aβ1-42 levels in the controls were significantly and positively correlated with MK and RK values in their left insula and MK and RK values in their bilateral OFC (Table 2). No significant correlation was noted between BAI scores and DKI parameters in the patients with FM. The VAS scores of the patients with FM were significantly correlated with AK values in their left amygdalae (left: rho = −0.603, p = 0.022; right: rho = −7.04, p = 0.005).

**Discussion**

We found that the patients with FM showed significant decreases in the DKI parameters of the left DLPFC. A PET-based study found that increased level of a glial activation marker, the [11 C]-PBR28 signal indicating that neuroinflammation with specifically microgliosis at the primary somatosensory cortex (S1), primary motor cortex, DLPFC, superior parietal lobule, supramarginal gyrus, supplementary motor area, posterior cingulate cortex, and dorsomedial prefrontal cortex in the patients with FM(14). The decrease in DKI parameters of the DLPFC in our study is compatible with the results of the PET study. We suggested that DKI could be an alternative imaging tool in examining the neuroinflammation state of patients with FM. Neuroinflammation can result in axon damage or demyelination and secondary structural changes. While both PET and DKI may detect neuroinflammation in the brain, the two modalities reflect different mechanisms of inflammation. The PET-based study can visualize the specific area in which glial or astrocyte activation. By contrast, DKI is based on the diffuse ability of water, mainly associated with the focal microstructural derangement. Further study is advised to perform these two modalities simultaneously (PET/MRI) to explore the detailed pathomechanism of neuroinflammation.

Our study is the first to apply DKI to examine the microstructural changes involving neuroinflammation in the patients with FM. In addition to the left DLPFC

showing significant decreases in all three DKI parameters (MK, RK, and AK), we found significant decreases in some DKI parameters at other areas such as the right DLPFC bilateral OFC. The DLPFC is associated with executive functions and contributes to pain inhibition, and OFC is involved in sensory integration and representing the affective value of reinforcers and expectation(15). Furthermore, different DKI parameters may reflect different types of information over time during neuroinflammation. An immunohistochemical study showed that an increased MK value is associated with increased astrogliosis(16). Another study reported that microgliosis is associated with increases in MK and RK values during acute inflammatory demyelination, however, the MK value decreases during the demyelination recovery period after cuprizone-induced demyelination(11).

A study utilized integrated PET/MRI to investigate the neuroinflammatory signatures in different chronic pain conditions including chronic back pain (CLBP) and FM. An elevation of 18kDa translocator protein (TSPO) level, which was expressed by activated glial cells, was observed in the brain of patients with CLBP and FM. TSPO signal elevated in the thalamus of CLBP patients in two independent cohorts, while TSPO signal elevations in patients with FM showed little involvement of thalamus(17). Consistently, we found that DKI parameters in thalamus did not show significant changes, suggesting that neuroinflammation rarely affects thalamus in FM. Another

study compared the difference between the patients with FM and complex regional pain syndrome (CRPS) by measuring the distribution volume ratio (DVR) of [11C]-(R)-PK11195 PET, representing the neuroinflammation level in the brain. A higher neuroinflammation levels at the left pre- and post-central gyri of patients with FM; while higher neuroinflammation levels were observed at the medulla, left insula, left thalamus, left superior temporal gyrus, bilateral putamen, and bilateral medial orbital gyri in patients with CRPS(18). Compatible with the study, we found no significant decrease in DKI parameters at the insula in patients with FM.

Tau and Aβ proteins are involved in the pathomechanism of neurological degeneration and can serve as biomarkers for neuron damage. Tau plays a prominent role in the stability of axonal microtubules. Tau pathology is associated with neuroinflammation. Depression and anxiety disorders are common in people with FM. The depression severity is associated with tau accumulation in specific brain regions, including the entorhinal cortex and inferior temporal area(19). We previously showed that the serum levels of Tau and Aβ1-42 significantly increased in patients with FM(20). We hypothesize that patients with FM could have a higher burden of regional neuronal damage associated with neuroinflammation. The present study found that a lower MK value is associated with a significantly higher serum Aβ1-42 level. Aβ1-42 is associated with phosphatidylcholine and sphingomyelin synthesis. Aβ1-42 oligomers can inhibit

myelin formation. Our data suggested that the increase in the Aβ1-42 level might be associated with regional impaired myelination and neuronal damage throughout neuroinflammation.

We found that the anxiety score and the AK value in the left insula were negatively correlated. An fMRI study suggested that the mesolimbic dopamine system regulates the pain inhibitory system in patients with chronic back pain(19). However, under depression and anxiety, the dopamine response to painful stimuli is insufficient(21). PET study found neuroinflammatory markers and stress scores were correlated in the left medial and superior frontal and left amygdala. The higher stress level was correlated with higher neuroinflammation states in patients with FM, suggesting that stress may trigger neuroinflammation in patients(18). Neuroinflammation in the patient with a major depressive episode (MDE) was examined by TSPO showed a significant increase in the insula, PFC and ACC compared to healthy controls(22). Abnormal amygdala activation to pain-related fear linked to treatment outcomes of FM(23). Microglia activation and proinflammatory cytokine production in the basolateral amygdala have been found to enhance presynaptic glutamate release associated with anxiety- and depression-like behaviours(24). We speculate that neuroinflammation specifically influences the mesolimbic dopamine system in patients with FM, leading to the dysfunction of the pain inhibitory system and mood regulation.

Our study has some limitations. First, the sample size was small, and it comprised Chinese women alone. Second, the cause-and-effect relationship could not be established because this was a cross-sectional study. Third, we did not compare our findings to patients with other chronic pain disorders. Fourth, we only investigated the serum levels of tau and Aβ1-42 as neuroinflammation biomarkers. Investigations on other biomarkers of neuroinflammation may aid in delineating the neuroinflammation characteristics of patients with FM. Finally, the present study showed different mean ages of FM patients and controls (50.6 vs 56.7 years old, respectively). The DKI has been investigated in the age-related change in the prefrontal cortex. They found no significant correlations between the DKI parameters over the age range between 47-62 years old(25). The age difference between the two groups might not affect our results significantly.

## Conclusion

The patients with FM had significant alterations in the DKI parameters of the DLPFC and OFC compared with the controls. These findings support site-specific neuroinflammation associated with microstructural changes in patients with FM.

## Acknowledgement

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**Disclosure statement**

The authors have declared no conflicts of interest.

**Legends of Figures**

**FIG. 1. (A)** Six pairs of targeted regions of interest (ROIs) with the coordinates in the sagittal and the coronal view. Colours are used to label the ROIs: amygdala (yellow), dorsal–lateral prefrontal cortex (DLPFC; red), hippocampus (light blue), insula (blue), orbital–frontal cortex (OFC; green), and thalamus (orange). **(B)** Differences in the axial kurtosis (AK), mean kurtosis (MK), and radial kurtosis (RK) values of the targeted regions of interest (ROIs) between the control and FM groups. ΔAK calculated as ⟦Control⟧_AK-⟦FM⟧_AK, where ⟦Control⟧_AK and ⟦FM⟧_AK is the average AK values in the control and FM groups, respectively. Similar formulas are applied to obtain ΔMK and ΔRK. **(C)** Bilateral dorsal–lateral prefrontal cortex (DLPFC) and bilateral orbital–frontal cortex (OFC) showing significantly lower MK values in the FM group than in the control group. The left DLPFC of the FM group showed a significant lower AK value than did that of the control group. Moreover, bilateral DLPFC and bilateral OFC showed a significantly lower RK value in the FM group than in the control group.

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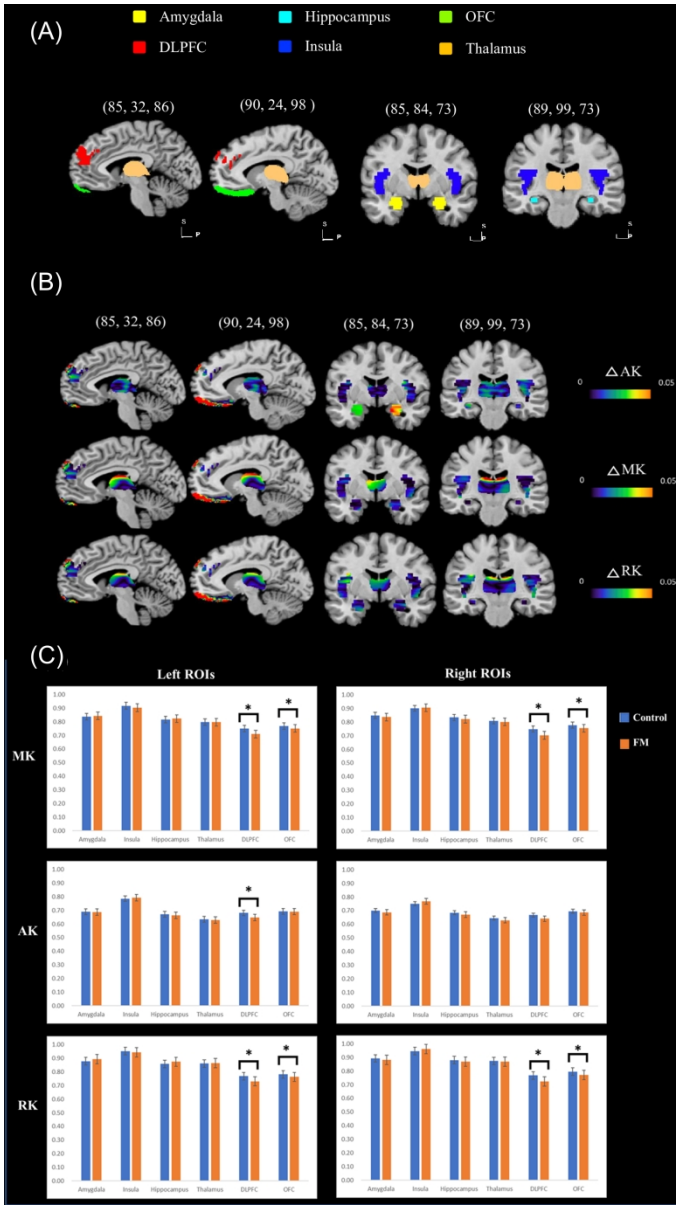


FIG. 1. (A) Six pairs of targeted regions of interest (ROIs) with the coordinates in the sagittal and the coronal view. Colours are used to label the ROIs: amygdala (yellow), dorsal–lateral prefrontal cortex (DLPFC; red), hippocampus (light blue), insula (blue), orbital–frontal cortex (OFC; green), and thalamus (orange). (B) Differences in the axial kurtosis (AK), mean kurtosis (MK), and radial kurtosis (RK) values of the targeted regions of interest (ROIs) between the control and FM groups. ΔAK calculated as 〖Control〗_AK-〖FM〗_AK, where 〖Control〗_AK and 〖FM〗_AK is the average AK values in the control and FM groups, respectively. Similar formulas are applied to obtain ΔMK and ΔRK. (C) Bilateral dorsal–lateral prefrontal cortex (DLPFC) and bilateral orbital–frontal cortex (OFC) showing significantly lower MK values in the FM group than in the control group. The left DLPFC of the FM group showed a significant lower AK value than did that of the control group. Moreover, bilateral DLPFC and bilateral OFC showed a significantly lower RK value in the FM group than in the control group.

579x1035mm (150 x 150 DPI)

## Tables

**TABLE 1.** Participant demographic characteristics and clinical profiles

| | **Control group (n = 14)** | **FM group (n = 14)** | ***p*** |
|---|---|---|---|
| **Age**, mean ± SD, years | 50.6 ± 7.6 | 56.9±7.2 | 0.034 |
| **BMI**, mean ± SD, kg/m$^2$ | 25.6 ± 4.8 | 23.5 ± 4.5 | 0.249 |
| **Clinical Profiles** | | | |
| BAI score | 4.2 ± 3.6 | 13.4 ± 8.9 | 0.001 |
| BDI-II score | 3.4 ± 5.9 | 11.4 ± 8.6 | 0.003 |
| Tau level, pg/mL | 23.54 ± 4.01 | 23.23 ± 3.68 | 0.835 |
| Aβ1-42 level, pg/mL | 16.52 ± 0.39 | 17.05 ± 0.45 | 0.003 |
| VAS | - | 5.4 ± 2.2 | - |

BMI: Body mass index; n: Number of participants; SD: Standard deviation; FM: Fibromyalgia; BAI: Beck's Anxiety Inventory; BDI-II: Beck's Depression Inventory-II; Aβ1-42: Amyloid β protein fragment 1-42; Visual Analog Scale: VAS.

Table 2 Spearman's rho correlation coefficients between DKI indexes in targeted ROIs

| ROI | DKI index | **FM (n = 14)** | | | **Controls (n = 14)** | | |
|---|---|---|---|---|---|---|---|
| | | Ab-42 | BAI | BDI-II | Ab-42 | BAI | BDI-II |
| Left insula | MK | Rho = 0.196, P = 0.503 | Rho = -0.233, P = 0.422 | Rho = -0.244, P = 0.401 | **Rho = -0.659, P = 0.014*** | Rho = -0.083, P = 0.779 | Rho = -0.100, P = 0.735 |
| | AK | Rho = 0.125, P = 0.670 | Rho = -0.064, P = 0.828 | Rho = -0.007, P = 0.982 | Rho = 0.253, P = 0.405 | **Rho = 0.521, P = 0.049*** | Rho = -0.465, P = 0.094 |
| | RK | Rho = -0.125, P = 0.657 | Rho = 0.035, P = 0.905 | Rho = -0.171, P = 0.560 | **Rho = -0.577, P = 0.039*** | Rho = -0.129, P = 0.659 | Rho = -0.377, P = 0.185 |
| Left DLPFC | MK | **Rho = -0.648, P = 0.012*** | Rho = 0.075, P = 0.799 | Rho = 0.430, P = 0.125 | Rho = 0.170, P = 0.578 | Rho = 0.455, P = 0.102 | Rho =- 0.011, P = 0.970 |
| | AK | **Rho = -0.675, P = 0.008*** | Rho = -0.138, P = 0.637 | Rho = -0.156, P = 0.593 | Rho = 0.181, P = 0.553 | Rho = 0.449, P = 0.108 | Rho = 0.073, P = 0.804 |
| | RK | **Rho = -0.640, P = 0.014*** | Rho = 0.139, P = 0.636 | Rho = 0.529, P = 0.052 | Rho = 0.143, P = 0.642 | Rho = -0.480, P = 0.082 | Rho = -0.053, P = 0.857 |
| Left OFC | MK | Rho = -0.191, P = 0.513 | Rho = 0.110, P = 0.708 | Rho = 0.277, P = 0.338 | **Rho = 0.588, P = 0.035*** | Rho = 0.105, P = 0.721 | Rho = 0.385, P = 0.174 |
| | AK | Rho = -0.214, P = 0.443 | Rho = -0.131, P = 0.643 | Rho = 0.099, P = 0.726 | **Rho = 0.637, P = 0.019*** | Rho = -0.192, P = 0.511 | Rho = -0.142, P = 0.629 |
| | RK | Rho = -0.02, P = 0.982 | Rho = 0.022, P = 0.940 | Rho = 0.144, P = 0.623 | Rho = 0.302, P = 0.316 | Rho = 0.080, P = 0.785 | Rho = 0.126, P = 0.667 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Right OFC | MK | Rho = 0.007, P = 0.982 | Rho = 0.055, P = 0.852 | Rho = 0.374, P = 0.187 | **Rho = 0.599, P = 0.031*** | Rho = 0.100, P = 0.733 | Rho = 0.155, P = 0.597 |
| | AK | Rho = 0.154, P = 0.616 | Rho = -0.011, P = 0.971 | Rho = 0.383, P = 0.197 | **Rho = 0.692, P = 0.009*** | Rho = -0.246, P = 0.397 | Rho = -0.268, P = 0.354 |
| | RK | Rho = 0.002, P = 0.994 | Rho = -0.026, P = 0.929 | Rho = 0.266, P = 0.358 | Rho = 0.055, P = 0.859 | Rho = 0.083, P = 0.779 | Rho = -0.033, P = 0.910 |

**ROI** region of interest**; DKI** diffusion kurtosis imaging**; DLPFC** dorsal lateral prefrontal cortex**; OFC** orbitofrontal cortex**; Ab-42** beta-amyloid-42 **FM** fibromyalgia**; BAI** Beck's Anxiety Inventory**; BDI-II** Beck's Depression Inventory, version II**.**
P corrected p values using Bonferroni correction for multiple testing (false discovery rate, FDR < 0.05).
*significant difference after Bonferroni correction for multiple testing ($p < 0.05$).