Association Between the C4 Binding Protein Level and White Matter Integrity in Major Depressive Disorder

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Objective Considerable evidence suggests that neuroinflammation plays an important role in the pathophysiology of major depressive disorder (MDD). However, the relationship between serum C4 binding protein (C4BP) and white matter (WM) tract integrity in MDD has not been investigated.

Methods We obtained diffusion tensor images of 44 patients with MDD and 44 healthy controls and performed TRActs Constrained by Underlying Anatomy (TRACULA) analysis to assess WM tract integrity. Serum C4-binding protein alpha chain (C4BPA) and C4-binding protein beta chain (C4BPB) levels were measured and in-between group comparisons were obtained. The correlation between serum C4BP levels and WM tract integrity was examined.

Results In comparison to healthy controls, both serum C4BPA and C4BPB were higher in MDD. Also, fractional anisotropy (FA) was increased in the left cingulum-angular bundle (CAB) in MDD, but not healthy controls (HCs). A significant correlation was found between serum C4BP and FA levels in the right cingulum-cingulate gyrus bundle (CCG) in MDD.

Conclusion This study is the first to investigate the correlation between serum C4BP levels and WM tract integrity in MDD. We identified an increase in WM integrity in the left CAB region in MDD. Furthermore, serum C4BP levels were higher in MDD, and this finding correlated with increased WM integrity in the right CCG region.

Keywords Major depressive disorder; Complement; C4 binding protein; Neuroinflammation; White matter tract integrity.

INTRODUCTION

Major depressive disorder (MDD) is a prevalent and recurrent psychiatric disorder that contributes to the leading cause of years lived with disability and is estimated to become the most debilitating disorder worldwide by 2030.1 Complex interactions between genetic, environmental, and psychological factors are known to predispose and affect the pathogenesis of depression, but the exact etiology remains unclear.

Recently, the neuroinflammation hypothesis of depression has received increasing attention due to the pivotal role of the inflammatory response in the development of depression.2,3 Stress-associated neurotoxic changes in the brain have been suggested to induce heightened inflammatory response, which in turn recruits various proinflammatory cytokines and other metabolites from the inflammatory process.4,5 Indeed, recent meta-analyses have shown significantly higher levels of serum interleukin (IL)-3, IL-6, IL-12, IL-18, sIL-2R, TNF-α, and CRP in MDD compared to healthy controls (HCs).5-8

The complement system is mainly composed of the classical, lectin, and alternate pathway and serves an important role in both inflammation and innate immunity. Whereas proper activation of this system enables the host to defend against pathogens, clears apoptotic and necrotic cells, and develop necessary antibody responses, dysregulation can otherwise lead to various pathogenic conditions.9 Therefore, strict regulation through numerous membrane-bound or soluble proteins is crucial for maintaining the optimal balance.9,10 In fact,
Taken together, these findings imply that complement activa-
tion markers such as CRP and IL-6 in patients with MDD. 23-25

Hippocampus volume and increased level of inflammatory
cells.21,22 These findings indicate that alterations in C4BP levels
have been found in patients with MDD than in HC.14-18

Increased levels of TNF-α was found to be associa-
ted with hyperactivity in the dorsal anterior cingulate cor-
tex and anterior insula, which are areas of the brain known for
processing negative affect.26 In relation to these findings, neuro-
imaging measures such as structural and functional MRI are
effective tools that can be used to investigate whether a height-
ened neuroinflammatory state, represented by increased C4BP
levels, accompanies alterations in the connectivity of neural
circuits in patients with depression.

In this study, diffusion tensor imaging (DTI) was used to
examine the relationship between serum C4BP levels and
structural changes in the white matter (WM) tracts of MDD
patients. We hypothesized that C4BP levels would increase
in patients with MDD and that higher C4BP levels would be
associated with alterations in the connectivity of different re-
gions of the brain in depression.

METHODS

Participants

A total of 88 participants, including 44 patients with MDD
and 44 HCs, were included in our study. Forty-four patients
with MDD were recruited from the outpatient psychiatric
clinic of Korea University Anam Hospital, Kyunghee Univer-
sity Hospital, and the National Center for Mental Health
(Seoul, Republic of Korea) between December 2018 and
April 2020. MDD patients aged between 18–64 years, and
currently in either euthymic or depressive state were included
in the study. MDD was diagnosed through structured clinical
interviews by four board-certified psychiatrists (B. J. Ham, K.
M. Han, J. W. Paik, and S. H. Lee) using the Diagnostic and
Statistical Manual of Mental Disorders, Fourth Edition, Text
Revision Axis I disorders. Patients with other comorbid major
psychiatric diseases, ongoing psychosis (i.e., hallucinations or
delusions), in high risk of suicide, having a past history of se-
vere medical illness, having a comorbid primary neurological
illness, currently under pregnancy or nursing, having abnor-
mal results on laboratory finding or physical examination, or
contraindicated to MRI were all excluded.

A total of 44 healthy participants were recruited using adver-
sisements for our HC group. Participants who went through
structured clinical interviews conducted by two board-certifi-
ced psychiatrists (K. M. Han and B. J. Ham) and confirmed
to have no ongoing or past history of Axis I or II disorders
were included in the study. The same exclusion criteria were
applied to both diagnostic groups, and the depression severity
was assessed using the 17-item Hamilton Depression Rating
Scale (HDRS) at the time of the MRI scan. Pharmacological
treatment was provided to all patients diagnosed with MDD
in this study at the start of enrollment. Further information re-
garding patient demographics and concurrent psychiatric
medications are summarized in Table 1.

The protocol of the present study was approved by the In-
stitutional Review Board (IRB) of the Korea University Anam
Hospital (IRB No: 2015AN0009). Each participants were
provided with a thorough explanation of the study and wrote
a contract agreeing for informed consent. All study methods
were consistent with former certified guidelines and the Decla-
ration of Helsinki.

Acquisition of C4BPA and C4BPB

Plasma sample preparation

After fasting for at least 12 h, 8 mL of peripheral blood was
drawn from all participants and collected in additive-free va-
cutainer tubes to be left at 37°C for 30 min. Centrifugation of
samples were done at 1,000 g for 15 min, then stored in parts
of 50 µL at -72°C until further analysis.

A multiple-affinity removal column (MARS 14) was used to
remove C4BPA and C4BPB. Then, 40 µL portion of plasma
diluted 4-fold with proprietary “buffer A” was adminis-
tered into a MARS 14 depletion column on a binary HPLC
system (20A Prominence, Shimadzu, Tokyo, Japan). The free
fraction was collected in a collection tube containing 100 µL of 5% SDS in 100 mM TEAB solution and then completely dried using a speed-vac concentrator (Thermo Fisher Scientific, Waltham, MA, USA). The dried sample was resuspended in 100 µL of 50 mM TEAB solution and sonicated for 10 min. Then, reduction (DTT, 10 mM, 56°C, 30 min) and alkylation (IAA, 20 mM, room temperature in the dark, 30 min) was done for 100 µg proteins. Afterwards, sample preparation was done by suspension-trapping (S-Trap)-based tryptic digestion based on the product guideline, with slight modifications. Once being loaded, 90%:10% methanol/50 mM ammonium bicarbonate and 50 mM ammonium bicarbonate was used to wash and digest samples, respectively. Trypsin (1:25 trypsin/protein) was added to the sample and overnight incubation was done at 37°C. The digested peptides were eluted by centrifuging at 1,000 g for 60 s. An additional elution was performed with 0.2% formic acid and 0.2% formic acid in 50% acetonitrile. The elutions were merged and vacuum-centrifuged to be dried and stored at -80°C until use.

Multiple Reaction Monitoring-Mass Spectrometry (MRM-MS) based confirmational study

Two different sets of plasma samples were analyzed using mass spectrometry based on the data-independent acquisition mode with capillary flow liquid chromatography in a short gradient. One patient was obtained from HC (44 cases), and the other was from patients diagnosed with MDD (44 cases). Peptides representing C4BPA and C4BPB were synthesized for the MRM-MS confirmation study. Peptide selection depended on the presence of the tryptic end, and no modification sites were found within 8–15 amino acids. The identification of peptides in protein targets was done using BLASTP and NCBI BLAST (www.ncbi.nlm.nih.gov/blast). The synthetic peptide analogs were LSLEIEQLELQR for C4BPA and ALLAFQESK for C4BPB. The incorporated lysine and arginine of each peptide were 13C heavy isotope-substituted peptides to differentiate them from the endogenous peptides.

A triple-quadrupole linear ion trap in the MRM mode was used to conduct MRM-MS runs were for preestablished transitions. At 15 µL/min flow rate, each sample (~5 µg) was injected into a reversed-phase HALO C18 column (Advanced Materials Technology, Inc., Wilmington, DE, USA) (10 cm×500 µm) using an Eksigent micro-UPLC system (AB Sciex, Foster City, CA, USA). Before usage, we equilibrated the column with 98% buffer A (0.1% formic acid in water) and 2% buffer B (0.1% formic acid in acetonitrile). For more than 40 min, a linear gradient of 2%–25% buffer B was used to elute peptides from the plasma with synthetic standard peptides. Electrospray MS data was collected using Turbo V Source on a 5,500 Q TRAP hybrid triple quadrupole/linear ion trap instrument (AB Sciex), and Analyst software 1.4.2 (Intelli-Quan algorithm) was applied for the integration of peaks. To increase specificity, MRM transitions were obtained at unit resolution for the Q1 and Q3 quadrupoles, and the temperature and voltage were set at 350°C and 5,500 V, respectively. The declustering potential was set at 120 V and the entrance potential was set at 10 V. The curtain gas was set at 30, and the collision gas was set at medium. For every transition, the scan time was 20 ms, and 5 ms was set as the holding time between the transition scans.

Quantification of serum protein level

Plasma concentrations of specific proteins were measured in the same sample set as a confirmatory study. By considering uniqueness and length, we selected LSLEIEQLELQR and ALLAFQESK as MRM-MS target peptides for C4BPA and C4BPB, respectively. The MRM-MS transition and scan parameters were optimized using the synthesized standard peptides, and individual protein levels were determined.

MRI data acquisition

All study participants underwent MRI scans, in which three-dimensional structural brain images were obtained using the 3.0-Tesla Trio whole-body imaging system (Siemens Healthcare GmbH, Erlangen, Germany). The parameters used to obtain DTI images are as follows: echo time, 84 ms; repetition time=6,300 ms; field of view, 230 mm; matrix, 128×128; slice thickness, 3 mm; orientation, transverse; diffusion directions, 20; voxel size, 1.8 mm×1.8 mm×3.0 mm; number of slices, 50; number of B0 images, 1; b-values, 0 and 600 s/mm²; acceleration factor (iPAT-GRAPPA), 2, with 38 reference lines for phase encoding direction and 6/8-phase partial Fourier.

Image processing

We performed a TRActs Constrained by UnderLying Anatomy (TRACULA) analysis using the protocol of a previous study27 to process and reconstruct the DTIs acquired from study participants. DTIs were initially registered to b=0 images, and FreeSurfer’s bbregister was used for the registration transformation.28 The FSL Bayesian estimation of diffusion parameters and FreeSurfer were used to map and reconstruct the segmentation of cortical and subcortical structure in each participant’s DTIs.29 The regional diffusion orientation of individual participants was obtained by ball-and-stick model of diffusion and probability distribution for the 18 major WM tracts was conducted using TRACULA. The following 18 WM tracts were included: anterior thalamic radiation (ATR), cingulum-angular bundle (CAB), cingulum-cingulate gyrus bundle (CCG), corticospinal tract (CST), the forceps major and forceps minor of the corpus callosum, inferior longitudinal fasciculus (ILF), superior longitudinal fasciculus-parietal bun-
dle (SLFp), superior longitudinal fasciculus-temporal bundle (SLFt), and uncinate fasciculus (UF) in both hemispheres. The FSL's DTIFit function (http://www.fmrib.ox.ac.uk/fsl) was used to obtain DTI parameters including fractional anisotropy (FA), axial diffusivity (AD), mean diffusivity (MD), and radial diffusivity (RD). Throughout the whole process, two trained researchers (W. S. Tae and Y. B. Kang) participated in the visual inspection of DTI measures.

Statistical analysis

IBM SPSS Statistics for Windows (version 24.0; IBM Corp., Armonk, NY, USA) was used for all statistical analyses. Diagnostic group differences in demographic and clinical characteristics were acquired using independent t-test and chi-square test. Group differences in serum C4BPA and C4BPB levels were analyzed by one-way analysis of covariance (ANCOVA), adjusted for age and sex.

The FA values extracted from 18 WM tracts were compared between MDD and HC using ANCOVA, with age and sex included as covariates. To determine the relationship between the mean values of serum protein and DTI scalar values, a two-tailed Pearson's partial correlation analysis was performed for serum C4BPA, C4BPB and FA, MD, RD, and AD values in the MDD group. To correct for the multiple comparison error in the analysis of 18 different WM tracts, Bonferroni's correction was performed with p<0.00278 as the level of significance (p<0.05/18 comparisons in each hemisphere) for all main analyses.

RESULTS

Demographic and clinical characteristics of participants

Table 1 summarizes the demographic and clinical characteristics of 44 MDD patients (14 males and 30 females; mean age, 37.61±12.85 years) and 44 healthy controls (13 male and 31 female; mean age, 37.36±12.44 years). There was no group difference regarding age, sex, and educational level. HDRS-17 scores were significantly higher in MDD (t=21.636, p<0.001) compared to HC. The mean duration of illness was 50.66±90.05 months in MDD patients at the time of the scan. Among the 44 MDD patients, 30 were drug-naïve, and 14 were on medication.

Association between C4BP levels and depression

Consistent with the discovery data, a statistically significant up-regulation of C4BPA and C4BPB was observed in MDD compared to HC (Table 1). The median plasma protein value for patients with MDD was 29.85±0.77 for C4BPA and 26.57±0.76 for C4BPB, while the controlled group showed median values of 28.05±1.01 and 24.77±1.05, respectively.

Group differences in the DTI parameters

Among the 18 major WM tracts, patients with MDD showed increased FA in the left CAB (F=10.597, p=0.002) compared

Table 1. Demographic and clinical characteristics of patients with MDD and HC

| Characteristics                  | MDD (N=44)          | HC (N=44)          | Significance (p) |
|----------------------------------|---------------------|-------------------|-----------------|
| Age (yr)                         | 37.61±12.85         | 37.36±12.44       | 0.926 (t=0.093) |
| Sex (F/M)                        | 30/14               | 31/13             | 0.817 (χ²=0.053) |
| Education level                  |                     |                   |                 |
| Elementary and middle school     | 1                   | 7                 |                 |
| High school or college/university| 40                  | 35                | 0.081 (χ²=5.033) |
| Above graduate school            | 3                   | 2                 |                 |
| HDRS-17 score                    | 19.05±5.47          | 0.59±1.44         | <0.001 (t=21.636) |
| Duration of illness (mon)        | 50.66±90.05         | NA                | NA              |
| Drug-naïve/medicated patients    | 30/14               | NA                | NA              |
| Medication (N)                   | 14                  | NA                | NA              |
| SSRI                             | 5                   | NA                | NA              |
| SNRI                             | 5                   | NA                | NA              |
| Other AD                         | 0                   | NA                | NA              |
| Combination of ADs               | 4                   | NA                | NA              |
| C4BPA                            | 29.85±0.77          | 28.05±1.01        | 89.99* (p<0.001) |
| C4BPB                            | 26.57±0.76          | 24.77±1.05        | 86.06* (p<0.001) |

Significance was examined using independent t-test, chi-squared test, and one-way analysis of covariance. *denotes significance. MDD, major depressive disorder; HC, healthy control; HDRS-17, 17-item Hamilton Depression Rating Scale; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin and norepinephrine reuptake inhibitor; combination of AD, combinations of two or more types of antidepressant; ADs, antidepressants; C4BPA, C4-binding protein alpha chain; C4BPB, C4-binding protein beta chain
to HCs (Table 2), and this result remained statistically significant even after Bonferroni's correction (p<0.00278). When applied to the other scalar values of DTI, patients with MDD also showed increased AD in the right CAB (F=5.882, p=0.017) and right UF (F=8.039, p=0.006) compared to HCs (Supplementary Table 1 in the online-only Data Supplement). However, the results did not survive Bonferroni's correction. There was no difference in MD and RD between the two groups (Supplementary Tables 2 and 3 in the online-only Data Supplement).

Correlation between HDRS-17 scores and FA values in the CAB region in depression

We performed partial correlation analysis to investigate the association between FA values in the CAB regions and HDRS-17 scores in patients with MDD, adjusted for age and sex. There was a positive trend towards significance between both CAB regions and HDRS-17 scores in MDD patients (left CAB: r[39]=0.305, n=44, p=0.053; right CAB: r[39]=0.270, n=44, p=0.088). However, p values were not statistically significant.

Correlation between DTI parameters and serum C4BP levels in depression

The FA values in the right CCG (C4BPA: r=0.496, p=0.001; C4BPB, r=0.444, p=0.004) showed significant correlation with serum C4BPA and C4BPB levels in patients with MDD (Table 3). On the other hand, significant inverse correlations were found between the MD values of the right CCG (C4BPA: r=-0.384, p=0.013; C4BPB: r=-0.353, p=0.024), right CST (C4BPA: r=-0.371, p=0.017; C4BPB: r=-0.371, p=0.017), and right SLFt (C4BPA: r=-0.356, p=0.022; C4BPB: r=-0.328, p=0.036) only in depressive patients, but not controls. RD values in patients with MDD were significantly inversely corre-

Table 2. Differences of FA values in the WM tracts between patients with MDD and HC

| WM tracts      | MDD           | HC            | F value | p     |
|----------------|---------------|---------------|---------|-------|
| Forceps major  | 0.589±0.027   | 0.594±0.041   | 1.574   | 0.213 |
| Forceps minor  | 0.475±0.036   | 0.493±0.038   | 0.619   | 0.433 |
| L ATR          | 0.420±0.034   | 0.425±0.028   | 4.310   | 0.041 |
| L CAB          | 0.378±0.052   | 0.376±0.032   | 10.597  | 0.002*|
| L CCG          | 0.564±0.043   | 0.568±0.036   | 0.485   | 0.488 |
| L CST          | 0.545±0.030   | 0.545±0.026   | 0.880   | 0.351 |
| L ILF          | 0.477±0.030   | 0.484±0.029   | 0.106   | 0.745 |
| L SLFp         | 0.429±0.024   | 0.441±0.027   | 0.122   | 0.727 |
| L SLFt         | 0.459±0.022   | 0.469±0.025   | 0.526   | 0.470 |
| L UF           | 0.426±0.031   | 0.436±0.027   | 1.523   | 0.221 |
| R ATR          | 0.419±0.034   | 0.424±0.032   | 0.329   | 0.568 |
| R CAB          | 0.411±0.051   | 0.401±0.042   | 2.225   | 0.139 |
| R CCG          | 0.582±0.037   | 0.571±0.047   | 2.153   | 0.146 |
| R CST          | 0.559±0.030   | 0.552±0.029   | 0.168   | 0.683 |
| R ILF          | 0.492±0.029   | 0.496±0.029   | 0.251   | 0.617 |
| R SLFp         | 0.447±0.028   | 0.453±0.030   | 0.121   | 0.729 |
| R SLFt         | 0.458±0.024   | 0.461±0.023   | 0.081   | 0.777 |
| R UF           | 0.450±0.037   | 0.454±0.025   | 4.337   | 0.040 |

Data are presented as mean±standard deviation. The F and p-values were obtained using one-way analysis of covariance adjusted for age and sex as covariates. The Bonferroni correction was applied in 18 WM tracts: 18 comparisons in both hemispheres, F<0.00278 (0.05/18). *denotes WM tracts that remained significant after Bonferroni correction. FA, fractional anisotropy; WM, white matter; MDD, major depressive disorder; HC, healthy control; L, left hemisphere; R, right hemisphere; ATR, anterior thalamic radiation; CAB, cingular-angulum bundle; CCG, cingulum–cingulate gyrus bundle; CST, corticospinal tract; ILF, inferior longitudinal fasciculus; SLFp, superior longitudinal fasciculus–parietal bundle; SLFt, superior longitudinal fasciculus–temporal bundle; UF, uncinate fasciculus

Table 3. Correlation between serum levels of C4BPA and C4BPB and FA values in MDD

| WM tracts      | C4BPA r | C4BPA p  | C4BPB r | C4BPB p  |
|----------------|---------|----------|---------|----------|
| Forceps major  | 0.089   | 0.582    | 0.116   | 0.471    |
| Forceps minor  | 0.214   | 0.179    | 0.193   | 0.227    |
| L ATR          | 0.125   | 0.435    | 0.124   | 0.441    |
| L CAB          | 0.042   | 0.793    | 0.033   | 0.837    |
| L CCG          | 0.342   | 0.029    | 0.336   | 0.032    |
| L CST          | 0.252   | 0.112    | 0.244   | 0.125    |
| L ILF          | 0.133   | 0.409    | 0.136   | 0.397    |
| L SLFp         | 0.166   | 0.301    | 0.176   | 0.271    |
| L SLFt         | 0.177   | 0.268    | 0.190   | 0.235    |
| L UF           | 0.302   | 0.055    | 0.274   | 0.083    |
| R ATR          | 0.125   | 0.435    | 0.127   | 0.430    |
| R CAB          | 0.048   | 0.767    | 0.046   | 0.774    |
| R CCG          | 0.496   | 0.001*   | 0.444   | 0.004    |
| R CST          | 0.289   | 0.067    | 0.240   | 0.130    |
| R ILF          | 0.072   | 0.654    | 0.045   | 0.780    |
| R SLFp         | 0.167   | 0.298    | 0.182   | 0.256    |
| R SLFt         | 0.321   | 0.041    | 0.325   | 0.038    |
| R UF           | 0.179   | 0.263    | 0.131   | 0.413    |

The r and p-values were obtained using Pearson’s correlation analysis including covariates for age and sex. The Bonferroni correction was applied in 18 WM tracts: 18 comparisons in both hemispheres, p<0.00278 (0.05/18). *denotes WM tracts that remained significant after Bonferroni correction. C4BPA, C4-binding protein alpha chain; C4BPB, C4-binding protein beta chain; FA, fractional anisotropy; WM, white matter; MDD, major depressive disorder; L, left hemisphere; R, right hemisphere; ATR, anterior thalamic radiation; CAB, cingular-angulum bundle; CCG, cingulum–cingulate gyrus bundle; CST, corticospinal tract; ILF, inferior longitudinal fasciculus; SLFp, superior longitudinal fasciculus–parietal bundle; SLFt, superior longitudinal fasciculus–temporal bundle; UF, uncinate fasciculus
lated with the left CCG (C4BPA: r=-0.371, p=0.017; C4BPB: r=-0.369, p=0.018), right CCG (C4BPA: r=-0.492, p=0.001; C4BPB: r=-0.511, p=0.001), and right SLF (C4BPA: r=-0.382, p=0.014; C4BPB: r=-0.359, p=0.021). Regarding AD values, significant inverse correlations were found in left ATR (C4BPA: r=-0.388, p=0.011; C4BPB: r=-0.371, p=0.016) and left CCG (C4BPA: r=-0.382, p=0.013; C4BPB: r=-0.360, p=0.019) in patients with MDD. When corrected for Bonferroni’s multiple comparison error, only the FA and RD values of the right CCG remained significantly associated with MDD compared with HC (Table 3 and Supplementary Tables 4-6 in the online-only Data Supplement).

**DISCUSSION**

This is the first study to investigate the correlation between serum C4BP levels and WM tract integrity in patients with MDD. We acquired serum C4BP data from all participants and investigated DTI-derived scalar values (FA, MD, RD, and AD) for the 18 WM tracts in patients with MDD using the TRACULA method. Significantly higher serum C4BP levels were found in patients with MDD. Moreover, we found increased FA values in the left CAB region in patients with MDD using DTI and TRACULA. Importantly, this analysis revealed for the first time that patients with MDD with higher serum C4BP levels showed increased FA in the right CCG region.

The increased FA value in the left CAB region in MDD patients is in line with the findings from previous studies in patients with treatment-resistant depression, posttraumatic stress disorder, and insomnia. The CAB directly connects the posterior cingulate cortex and subiculum of the hippocampus, both of which are part of the default mode network, which is altered in MDD. Furthermore, the integrity of the CAB is associated with decision-making, episodic memory, and executive control. Which, when disrupted, can cause depressive symptoms. Many studies have suggested a temporal relationship between hippocampal volume and duration of MDD. Although the current study focused on WM tract integrity and did not examine the volume of regional gray matter, the increased FA value in the CAB region may reflect WM rearrangement in the parahippocampal tracts to compensate for gray matter deficits in the hippocampal area in patients with MDD.

In accordance with our hypothesis, serum C4BPA and C4BP levels were higher in patients with MDD. C4BP is known for its role in mediating the cleavage of C3b, a key component necessary for myelin phagocytosis by microglia/macrophages in the CNS. When left uncontrolled, excessive phagocytosis of myelin may result in reduced WM integrity through demyelination, contributing to regional changes in the brain. This may be interpreted as the host’s immune response to protect myelin from excessive degradation, suggesting a compensatory role of C4BP in preventing dangerous complement activation that may accelerate the neurodegenerative process in the host, but allows the low level of complement activation required for enhanced clearance.

When applied to DTI scalar values, only the FA of the right CCG region showed significant correlation with serum C4BPA levels in patients with MDD. The RD of the same WM region showed an inverse correlation with serum C4BP levels. Increased FA along with decreased RD indicates greater myelination and WM integrity. This is interesting because most DTI studies have reported decreased FA values in the cingulum region of MDD patients. Unlike other WM tracts, previous studies have revealed prolonged cingulum maturation from adolescence up to the mid-20s or later, and the peak FA was found to be reached at a mean age of 42 years. This lengthy period of maturation may predispose the cingulum region to diverse changes in the orientation and branching of the WM tracts, resulting in controversial FA values. The increased FA may reflect a greater number of longitudinally aligned fibers in proportion to obliquely aligned fibers and greater myelination of WM fibers, or it may be the complex result of various physiological factors such as reduced axon diameter, decreased WM branching, and lower intra-voxel crossing. The positive correlation between serum C4BPA levels and FA values may imply greater myelination of WM fibers through the regulation of the classical pathway, in which myelin is spared from C3b opsonization by inhibiting of C3 convertase. In addition, this discrepancy may result from underlying crucial genetic polymorphisms that moderate microstructural integrity, such as in the case of brain-derived neurotrophic factor alleles in MDD patients, in which different FA values were achieved in the left cingulum (rostral) region depending on different genetic alleles.

Taken together, these results suggest that the complement pathway may play a role in the neuroinflammatory process of depression through the compensatory action of C4BP, promoting greater myelination, and hence, increased WM integrity in selective regions of the brain.

Our study had several limitations. First, this is a cross-sectional study, and therefore the causal directionality regarding the relationship between variables is difficult to infer. Future studies with a longitudinal design may clarify the relationship between variables and assist towards a much more accurate view in the pathophysiology underlying changes in WM integrity in MDD patients. In addition, the current study did not include other components of the complement system or serum inflammatory markers that could support our explanation of the results. Further studies that include various markers...
of the classical complement pathway or inflammatory markers, such as CRP and myelin, could provide a more holistic view regarding the action of complement system in MDD. Also, proteomic analysis in the current study was acquired from blood plasma samples rather than from CSF. Although CSF is a natural body fluid that accurately reflects live, ongoing processes in brain, the invasiveness of the procedure is a major barrier to study participation and method design, especially in studies involving large human samples. The accessibility and convenience of blood sampling, on the other hand, was one of the main reasons for choosing this type of sampling in the current study. However, further studies investigating C4BP levels in CSF of MDD patients may provide a more accurate view in the association between C4BP and WM integrity. In addition, our MDD sample was heterogeneous in terms of the types and doses of medication, which can affect serum C4BP levels and WM tract integrity. Moreover, this study did not control for the effect of variables such as number of episodes, childhood trauma, familial history, economic level, lifestyle, and dietary habits. Future studies should consider more consistent demographic and clinical variables.

In conclusion, we identified an increase in WM integrity in the left CAB region of individuals with MDD. Furthermore, MDD patients had higher levels of serum C4BP, which was correlated with increased WM integrity in the right CCG region. To our knowledge, this study is the first to investigate the correlation between serum C4BP levels and WM integrity using DTI and TRACULA methods in samples from unipolar depression. Our findings suggest a possible neuroprotective role for C4BP in compensating for proinflammatory process of MDD. Further investigations with larger sample sizes and more serum markers, and, if possible, CSF markers, are needed to obtain a clearer view of the role of the complement system in MDD.

Supplementary Materials
The online-only Data Supplement is available with this article at https://doi.org/10.30773/pi.2022.0100.

Availability of Data and Material
The datasets generated or analyzed during the study are available from the corresponding author on reasonable request.

Conflicts of Interest
Kyu-Man Han, a contributing editor of the Psychiatry Investigation, was not involved in the editorial evaluation or decision to publish this article. All remaining authors have declared no conflicts of interest.

Author Contributions
Conceptualization: Jihoon Park, Kyu-Man Han, Woo-Suk Tae, Byung-Joo Ham. Data curation: Jihoon Park, Youbin Kang, Un-Beom Kang, Hyesub Chu, Byung-Joo Ham. Formal analysis: Jihoon Park, Youbin Kang, Un-Beom Kang, Hyesub Chu, Byung-Joo Ham. Funding acquisition: Byung-Joo Ham. Investigation: Jihoon Park, Youbin Kang, Kyu-Man Han, Woo-Suk Tae, Un-Beom Kang, Byung-Joo Ham. Methodology: Youbin Kang, Kyu-Man Han, Woo-Suk Tae, Un-Beom Kang, Hyesub Chu, Byung-Joo Ham. Project administration: Byung-Joo Ham. Resources: Un-Beom Kang, Byung-Joo Ham. Software: Woo-Suk Tae, Un-Beom Kang. Supervision: Youbin Kang, Kyu-Man Han, Woo-Suk Tae, Un-Beom Kang, Byung-Joo Ham. Validation: all authors. Visualization: Youbin Kang, Un-Beom Kang, Byung-Joo Ham. Writing—original draft: Jihoon Park, Un-Beom Kang, Byung-Joo Ham. Writing—review & editing: Jihoon Park, Youbin Kang, Kyu-Man Han, Woo-Suk Tae, Byung-Joo Ham.

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REFERENCES
1. Vos T, Barber RM, Bell B, Bertozzi-Villa A, Biryukov S, Bolliger I, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015;386:743-800.
2. Berk M, Williams LJ, Jacka FN, O’Neill A, Pasco JA, Moylan S, et al. So depression is an inflammatory disease, but where does the inflammation come from? BMC Med 2013;11:200.
3. Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nat Rev Immunol 2016;16:22-34.
4. Kim YK, Won E. The influence of stress on neuroinflammation and alterations in brain structure and function in major depressive disorder. Behav Brain Res 2017;329:6-11.
5. Troubat R, Barone P, Leman S, Desmidt T, Cressant A, Atanasova B, et al. Neuroinflammation and depression: a review. Eur J Neurosci 2021;53:151-171.
6. Osimo EF, Pillinger T, Rodriguez IM, Khandaker GM, Pariante CM, Howes OD. Inflammatory markers in depression: a meta-analysis of mean differences and variability in 5,166 patients and 5,083 controls. Brain Behav Immun 2020;87:901-909.
7. Goldsmith DR, Rapaport MH, Miller BJ. A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder and depression. Mol Psychiatry 2016;21:1696-1709.
8. Köhler CA, Freitas TH, Maes M, de Andrade NQ, Liu CS, Fernandes BS, et al. Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. Acta Psychiatr Scand 2017;135:373-387.
9. Sjöberg AP, Trouw LA, McGrath FD, Hack CE, Blom AM. Regulation of complement activation by C-reactive protein: targeting of the inhibitory activity of C4b-binding protein. J Immunol 2006;176:7612-7620.
10. Blom AM, Kask L, Dahlbäck B. CCP1-4 of the C4b-binding protein alpha-chain are required for factor I mediated cleavage of complement factor C3b. Mol Immunol 2003;39:547-556.
11. Tenner AJ, Stevens B, Woodruff TM. New tricks for an ancient system: physiological and pathological roles of complement in the CNS. Mol Immunol 2018;102:3-13.
12. Lynch NJ, Willis CL, Nolan CC, Roscher S, Fowler MJ, Weihe E, et al.
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Microglial activation and increased synthesis of complement component C1q precedes blood-brain barrier dysfunction in rats. Mol Immunol 2004;40:709-716.

13. Cho K. Emerging roles of complement protein C1q in neurodegeneration. Aging Dis 2019;10:652-663.

14. Wei J, Liu Y, Zhao L, Yang X, Ni P, Wang Y, et al. Plasma complement component 4 increases in patients with major depressive disorder. Neuropsychiatr Dis Treat 2018;14:37-41.

15. Stelzhammer V, Haenisch F, Chan MK, Cooper JD, Steiner J, Steeb H, et al. Proteomic changes in serum of first onset, antidepressant-naive major depression patients. Int J Neuropsychopharmacol 2014;17:1599-1608.

16. Maes M, Delange J, Ranjan R, Meltzer HY, Desnyder R, Cooremans W, et al. Acute phase proteins in schizophrenia, mania and major depression: modulation by psychotropic drugs. Psychiatry Res 1997;66:1-11.

17. Boyle SH, Jackson WG, Suarez EC. Hostility, anger, and depression predict increases in C3 over a 10-year period. Brain Behav Immun 2007;21:816-823.

18. Morgan BP, Harris CL. Complement, a target for therapy in inflammatory and degenerative diseases. Nat Rev Drug Discov 2015;14:857-877.

19. Suankratay C, Mold C, Zhang Y, Lint TF, Gewurz H. Mechanism of complement-dependent haemolysis via the lectin pathway: role of the complement regulatory proteins. Clin Exp Immunol 1999;117:442-448.

20. Blom AM, Villoutreix BO, Dahlbäck B. Complement inhibitor C4b-binding protein-friend or foe in the innate immune system? Mol Immunol 2004;40:1333-1346.

21. Veerhuis R, Boshuizen RS, Familian A. Amyloid associated proteins in Alzheimer's and prion disease. Curr Drug Targets CNS Neurol Disord 2005;4:235-248.

22. Trouw LA, Nielsen HM, Minthion L, Londos E, Landberg G, Veerhuis R, et al. C4b-binding protein in Alzheimer's disease: binding to Aβ1-42 and to dead cells. Mol Immunol 2008;45:3649-3660.

23. Frödl T, Carballo A, Hughes MM, Saleh K, Fagan A, Skokauskas N, et al. Reduced expression of glucocorticoid-inducible genes GILZ and SGK1: high IL-6 levels are associated with reduced hippocampal volumes in major depressive disorder. Tran Psychiatry 2012;2:88.

24. Satizabal CL, Zhu YC, Mazoyer B, Dufouil C, Tzourio C. Circulating IL-6 and CRP are associated with MRI findings in the elderly: the 3C-Dijon Study. Neurology 2012;78:720-727.

25. Su L, Fahay YO, Hong YT, Fryer TD, Mak E, Gabel S, et al. Neuroinflammatory and morphological changes in late-life depression: the NIMROD study. Br J Psychiatry 2016;209:525-526.

26. Slavich GM, Way BM, Eisenberger NI, Taylor SE. Neural sensitivity to social rejection is associated with inflammatory responses to social stress. Proc Natl Acad Sci U S A 2010;107:14817-14822.

27. Lee SH, Coutou JP, Willkens P, Vendiki A, Rosas HD, Salat DH. Tract-based analysis of white matter degeneration in Alzheimer's disease. Neuroscience 2015;301:79-89.

28. Greve DN, Fischl B. Accurate and robust brain image alignment using boundary-based registration. Neuroimage 2009;48:63-72.

29. Behrens TE, Berg HJ, Jbabdi S, Rushworth MF, Woolrich MW. Probabilistic diffusion tractography with multiple fibre orientations: what can we gain? Neuroimage 2007;34:144-155.

30. Young CB, EP NR, Fernández G, Schene A. Decreased hippocampal volume is related to white matter abnormalities in treatment-resistant depression. Int J Brain Disord Treat 2016;2:012.

31. Averill CL, Averill LA, Wrocklage KM, Scott JC, Akiki TJ, Schweinsburg B, et al. Altered white matter diffusivity of the cingulum angular bundle in posttraumatic stress disorder. Mol Neuropsychiatry 2018;4:75-82.

32. Rostampour M, Girayrouz L, Rostampour N, Kaveh D, Noori K, Fadaei R, et al. Asymmetric alterations of white matter integrity in patients with insomnia disorder. Brain Imaging Behav 2022;16:389-396.

33. Heilbronner SR, Haber SN. Frontal cortical and subcortical projections provide a basis for segmenting the cingulum bundle: implications for neuroimaging and psychiatric disorders. J Neurosci 2014;34:10041-10054.

34. Bubb EJ, Metzler-Baddeley C, Aggleton JP. The cingulum bundle: anatomy, function, and dysfunction. Neurosci Biobehav Rev 2018;92:104-127.

35. Sripada RK, King AP, Welsh RG, Garfinkel SN, Wang X, Sripada CS, et al. Neural dysregulation in posttraumatic stress disorder: evidence for disrupted equilibrium between salience and default mode brain networks. Psychosom Med 2012;74:904-911.

36. Khalsa S, Mayhew SD, Checlaz M, Bagary M, Bagshaw AP. The structural and functional connectivity of the posterior cingulate cortex: comparison between deterministic and probabilistic tractography for the investigation of structure-function relationships. Neuroimage 2014;102 Pt 1:118-127.

37. Zhou HX, Chen X, Shen YQ, Li L, Chen NZ, Zhu ZC, et al. Rumination and the default mode network: meta-analysis of brain imaging studies and implications for depression. Neuroimage 2020;206:116287.

38. Andrews-Hanna JR, Reidler JS, Sepulcre J, Poulin R, Buckner RL. Functional-anatomical fractionation of the brain's default network. Neuron 2010;65:550-562.

39. Wu Y, Sun D, Wang Y, Wang Y, Ou S. Segmentation of the cingulum bundle in the human brain: a new perspective based on DSI tractography and fiber dissection study. Front Neuroanat 2016:10:84.

40. Ezzati A, Katz MJ, Lipton ML, Zimmerman ME, Lipton RB. Hippocampal volume and cingulum bundle fractional anisotropy are independently associated with verbal memory in older adults. Brain Imaging Behav 2016:10:652-659.

41. McKinnon MC, Yucel K, Nazarov A, MacQueen GM. A meta-analysis examining clinical predictors of hippocampal volume in patients with major depressive disorder. J Psychiatry Neurosci 2009;34:41-54.

42. Videbech P, Ravnikle B. Hippocampal volume and depression: a meta-analysis of MRI studies. Am J Psychiatry 2004;161:1957-1966.

43. Campbell S, Marriott M, Nahmias C, MacQueen GM. Lower hippocampal volume in patients suffering from depression: a meta-analysis. Am J Psychiatry 2004;161:598-607.

44. MacQueen GM, Campbell S, McEwen BS, Macdonald K, Amano S, Jofe RT, et al. Course of illness, hippocampal function, and hippocampal volume in major depression. Proc Natl Acad Sci U S A 2003;100:1387-1392.

45. Sheline YI, Sanghavi M, Mintun MA, Gado MH. Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. J Neurosci 1999;19:5034-5043.

46. Rawal N, Pangburn MK. Role of the C3b-binding site on C4b-binding protein in regulating classical pathway CS conversion. Mol Immunol 2007;44:1105-1114.

47. Dunkelberger JR, Song WC. Complement and its role in innate and adaptive immune responses. Cell Res 2010;20:34-50.

48. Merle NS, Church SE, Fremeaux-Bacchi V, Roumenina LT. Complement and its role in innate and adaptive immunity and psychiatric disorders. J Neurosci 2014;34:11071-11072.
continues from childhood into adulthood. J Neurosci 2011;31:10937-10947.

54. Lebel C, Gee M, Camicioni R, Wieler M, Martin W, Beaulieu C. Diffusion tensor imaging of white matter tract evolution over the lifespan. Neuroimage 2012;60:340-352.

55. Beaulieu C. The basis of anisotropic water diffusion in the nervous system—A technical review. NMR Biomed 2002;15:435-455.

56. Le Bihan D. Diffusion, perfusion and functional magnetic resonance imaging. J Mal Vasc 1995;20:203-214.

57. Schwartz ED, Hackney DB. Diffusion-weighted MRI and the evaluation of spinal cord axonal integrity following injury and treatment. Exp Neurol 2003;184:570-589.

58. Mole JP, Subramanian L, Bracht T, Morris H, Metzler-Baddeley C, Linden DE. Increased fractional anisotropy in the motor tracts of Parkinson’s disease suggests compensatory neuroplasticity or selective neurodegeneration. Eur Radiol 2016;26:3327-3335.

59. Carballedo A, Amico F, Ugwu I, Fagan AJ, Fahey C, Morris D, et al. Reduced fractional anisotropy in the uncinate fasciculus in patients with major depression carrying the met-allele of the Val66Met brain-derived neurotrophic factor genotype. Am J Med Genet B Neuropsychiatr Genet 2012;159B:537-548.
**Supplementary Table 1. Differences of axial diffusivity values in the WM tracts between MDD and HCs**

| WM tracts | MDD         | HC          | F value | p     |
|-----------|-------------|-------------|---------|-------|
|           | Mean        | SD          | Mean    | SD    |       |
| Forceps major | 1.36E-03   | 3.74E-05   | 1.37E-03 | 4.46E-05 | 0.279 | 0.598 |
| Forceps minor | 1.22E-03   | 3.60E-05   | 1.24E-03 | 4.58E-05 | 0.905 | 0.344 |
| L ATR     | 1.10E-03   | 3.17E-05   | 1.10E-03 | 3.29E-05 | 0.016 | 0.899 |
| L CAB     | 1.09E-03   | 4.53E-05   | 1.10E-03 | 6.74E-05 | 3.528 | 0.064 |
| L CCG     | 1.24E-03   | 5.19E-05   | 1.25E-03 | 6.12E-05 | 1.035 | 0.312 |
| L CST     | 1.20E-03   | 3.60E-05   | 1.20E-03 | 3.39E-05 | 0.332 | 0.566 |
| L ILF     | 1.24E-03   | 3.88E-05   | 1.23E-03 | 3.62E-05 | 0.559 | 0.457 |
| L SLFp    | 1.10E-03   | 3.53E-05   | 1.10E-03 | 3.08E-05 | 1.016 | 0.316 |
| L SLFt    | 1.15E-03   | 3.24E-05   | 1.15E-03 | 3.04E-05 | 0.604 | 0.439 |
| L UF      | 1.17E-03   | 3.40E-05   | 1.18E-03 | 3.29E-05 | 0.003 | 0.954 |
| R ATR     | 1.08E-03   | 2.94E-05   | 1.08E-03 | 3.34E-05 | 0.056 | 0.814 |
| R CAB     | 1.04E-03   | 5.68E-05   | 1.09E-03 | 1.38E-04 | 5.882 | 0.017*|
| R CCG     | 1.27E-03   | 5.39E-05   | 1.25E-03 | 4.17E-05 | 1.379 | 0.244 |
| R CST     | 1.17E-03   | 3.08E-05   | 1.18E-03 | 4.70E-05 | 3.692 | 0.058 |
| R ILF     | 1.22E-03   | 4.47E-05   | 1.23E-03 | 3.75E-05 | 1.820 | 0.181 |
| R SLFp    | 1.07E-03   | 3.62E-05   | 1.08E-03 | 3.25E-05 | 0.041 | 0.840 |
| R SLFt    | 1.10E-03   | 3.22E-05   | 1.10E-03 | 3.31E-05 | 0.000 | 0.983 |
| R UF      | 1.12E-03   | 2.77E-05   | 1.15E-03 | 6.62E-05 | 8.039 | 0.006*|

The F and p-values were obtained using one-way analysis of covariance adjusted for age and sex as covariates. The Bonferroni correction was applied in 18 WM tracts: 18 comparisons in both hemispheres, p<0.00278 (0.05/18). *denotes WM tracts that remained significant after Bonferroni correction. WM, white matter; MDD, major depressive disorder; HC, healthy control; SD, standard deviation; L, left hemisphere; R, right hemisphere; ATR, anterior thalamic radiation; CAB, cingular-angulum bundle; CCG, cingulum–cingulate gyrus bundle; CST, cortico-spinal tract; ILF, inferior longitudinal fasciculus; SLFp, superior longitudinal fasciculus–parietal terminations; SLFt, superior longitudinal fasciculus–temporal terminations; UF, uncinate fasciculus
Supplementary Table 2. Differences of mean diffusivity values in the WM tracts between MDD and HCs

| WM tracts   | MDD          | HC           | F value | p   |
|-------------|--------------|--------------|---------|-----|
|             | Mean         | SD           | Mean    | SD  |
| Forceps major| 7.60E-04     | 2.53E-05     | 7.63E-04| 2.65E-05| 0.180 | 0.673 |
| Forceps minor| 7.72E-04     | 2.57E-05     | 7.71E-04| 2.76E-05| 0.079 | 0.780 |
| L ATR       | 7.37E-04     | 2.82E-05     | 7.35E-04| 2.54E-05| 0.150 | 0.699 |
| L CAB       | 7.63E-04     | 3.97E-05     | 7.73E-04| 4.09E-05| 0.059 | 0.809 |
| L CCG       | 7.22E-04     | 3.53E-05     | 7.22E-04| 3.32E-05| 0.158 | 0.692 |
| L CST       | 7.18E-04     | 2.48E-05     | 7.18E-04| 2.43E-05| 0.981 | 0.325 |
| L ILF       | 7.89E-04     | 2.96E-05     | 7.81E-04| 3.00E-05| 0.104 | 0.748 |
| L SLFp      | 7.45E-04     | 2.95E-05     | 7.35E-04| 3.09E-05| 0.314 | 0.577 |
| L SLFt      | 7.50E-04     | 2.64E-05     | 7.41E-04| 2.91E-05| 0.002 | 0.967 |
| L UF        | 7.86E-04     | 2.81E-05     | 7.79E-04| 2.38E-05| 0.421 | 0.518 |
| R ATR       | 7.30E-04     | 2.55E-05     | 7.25E-04| 2.87E-05| 0.301 | 0.585 |
| R CAB       | 7.09E-04     | 4.92E-05     | 7.49E-04| 8.33E-05| 1.292 | 0.259 |
| R CCG       | 7.21E-04     | 3.05E-05     | 7.17E-04| 3.29E-05| 0.092 | 0.762 |
| R CST       | 6.91E-04     | 2.67E-05     | 7.01E-04| 3.47E-05| 0.230 | 0.633 |
| R ILF       | 7.70E-04     | 3.22E-05     | 7.74E-04| 2.92E-05| 0.862 | 0.356 |
| R SLFp      | 7.13E-04     | 2.77E-05     | 7.12E-04| 3.03E-05| 0.234 | 0.630 |
| R SLFt      | 7.18E-04     | 2.60E-05     | 7.17E-04| 2.68E-05| 0.026 | 0.872 |
| R UF        | 7.34E-04     | 2.91E-05     | 7.47E-04| 4.64E-05| 1.708 | 0.195 |

The F and p-values were obtained using one-way analysis of covariance adjusted for age and sex as covariates. The Bonferroni correction was applied in 18 WM tracts: 18 comparisons in both hemispheres, \( p < 0.00278 \) (0.05/18). WM, white matter; MDD, major depressive disorder; HC, healthy control; SD, standard deviation; L, left hemisphere; R, right hemisphere; ATR, anterior thalamic radiation; CAB, cingular-angulum bundle; CCG, cingulum–cingulate gyrus bundle; CST, corticospinal tract; ILF, inferior longitudinal fasciculus; SLFp, superior longitudinal fasciculus–parietal terminations; SLFt, superior longitudinal fasciculus–temporal terminations; UF, uncinate fasciculus
**Supplementary Table 3.** Differences of radial diffusivity values in the WM tracts between MDD and HCs

| WM tracts   |   | MDD       |       | HC        |       | F value | p    |
|-------------|---|-----------|-------|-----------|-------|---------|------|
|             |   | Mean      | SD    | Mean      | SD    |         |      |
| Forceps major|   | 4.62E-04  | 3.18E-05 | 4.60E-04  | 4.27E-05 | 0.427  | 0.515 |
| Forceps minor|   | 5.49E-04  | 3.67E-05 | 5.36E-04  | 3.88E-05 | 0.768  | 0.383 |
| L ATR       |   | 5.58E-04  | 3.61E-05 | 5.54E-04  | 3.09E-05 | 1.076  | 0.303 |
| L CAB       |   | 6.02E-04  | 5.18E-05 | 6.09E-04  | 3.67E-05 | 3.050  | 0.084 |
| L CCG       |   | 4.66E-04  | 4.32E-05 | 4.60E-04  | 3.62E-05 | 1.548  | 0.217 |
| L CST       |   | 4.75E-04  | 3.17E-05 | 4.75E-04  | 3.01E-05 | 1.226  | 0.271 |
| L ILF       |   | 5.65E-04  | 3.45E-05 | 5.55E-04  | 3.55E-05 | 0.037  | 0.848 |
| L SLFp      |   | 5.67E-04  | 3.12E-05 | 5.52E-04  | 3.62E-05 | 0.004  | 0.947 |
| L SLFt      |   | 5.51E-04  | 2.87E-05 | 5.38E-04  | 3.37E-05 | 0.261  | 0.611 |
| L UF        |   | 5.91E-04  | 3.45E-05 | 5.80E-04  | 2.93E-05 | 1.300  | 0.257 |
| R ATR       |   | 5.55E-04  | 3.44E-05 | 5.47E-04  | 3.58E-05 | 0.015  | 0.901 |
| R CAB       |   | 5.45E-04  | 5.64E-05 | 5.77E-04  | 6.37E-05 | 0.040  | 0.842 |
| R CCG       |   | 4.46E-04  | 3.78E-05 | 4.52E-04  | 4.72E-05 | 1.330  | 0.252 |
| R CST       |   | 4.51E-04  | 3.38E-05 | 4.61E-04  | 3.66E-05 | 0.026  | 0.872 |
| R ILF       |   | 5.44E-04  | 3.52E-05 | 5.44E-04  | 3.42E-05 | 0.224  | 0.637 |
| R SLFp      |   | 5.33E-04  | 3.15E-05 | 5.29E-04  | 3.57E-05 | 0.453  | 0.503 |
| R SLFt      |   | 5.27E-04  | 2.92E-05 | 5.23E-04  | 2.93E-05 | 0.039  | 0.844 |
| R UF        |   | 5.40E-04  | 3.88E-05 | 5.46E-04  | 4.18E-05 | 0.185  | 0.668 |

The F and p-values were obtained using one-way analysis of covariance adjusted for age and sex as covariates. The Bonferroni correction was applied in 18 WM tracts: 18 comparisons in both hemispheres, p=0.00278 (0.05/18). WM, white matter; MDD, major depressive disorder; HC, healthy control; SD, standard deviation; L, left hemisphere; R, right hemisphere; ATR, anterior thalamic radiation; CAB, cingular-angulum bundle; CCG, cingulum–cingulate gyrus bundle; CST, corticospinal tract; ILF, inferior longitudinal fasciculus; SLFp, superior longitudinal fasciculus–parietal terminations; SLFt, superior longitudinal fasciculus–temporal terminations; UF, uncinate fasciculus
**Supplementary Table 4.** Correlation between serum levels of C4BPA and C4BPB and mean diffusivity values in patients with MDD

| WM tracts       | C4BPA       | C4BPB       |
|-----------------|-------------|-------------|
|                 | r           | p           | r           | p           |
| Forceps major   | -0.162      | 0.311       | -0.122      | 0.449       |
| Forceps minor   | -0.064      | 0.693       | -0.057      | 0.721       |
| L ATR           | -0.326      | 0.037       | -0.296      | 0.061       |
| L CAB           | -0.259      | 0.103       | -0.227      | 0.154       |
| L CCG           | -0.318      | 0.043       | -0.306      | 0.052       |
| L CST           | -0.231      | 0.147       | -0.198      | 0.215       |
| L ILF           | -0.241      | 0.129       | -0.208      | 0.192       |
| L SLFp          | -0.186      | 0.244       | -0.156      | 0.331       |
| L SLFt          | -0.254      | 0.108       | -0.228      | 0.151       |
| L UF            | -0.138      | 0.388       | -0.136      | 0.398       |
| R ATR           | -0.219      | 0.170       | -0.206      | 0.195       |
| R CAB           | -0.159      | 0.319       | -0.171      | 0.286       |
| R CCG           | -0.384      | 0.013*      | -0.353      | 0.024*      |
| R CST           | -0.371      | 0.017*      | -0.371      | 0.017*      |
| R ILF           | -0.225      | 0.157       | -0.218      | 0.171       |
| R SLFp          | -0.304      | 0.053       | -0.276      | 0.081       |
| R SLFt          | -0.356      | 0.022*      | -0.328      | 0.036*      |
| R UF            | -0.084      | 0.603       | -0.103      | 0.521       |

The r and p-value were obtained using Pearson’s correlation analysis including covariates for age and sex. The Bonferroni correction was applied in 18 WM tracts: 18 comparisons in both hemispheres, p<0.00278 (0.05/18). *denotes WM tracts that remained significant after Bonferroni correction. MDD, major depressive disorder; C4BPA, C4b-binding protein alpha chain; C4BPB, C4b-binding protein beta chain; L, left hemisphere; R, right hemisphere; ATR, anterior thalamic radiation; CAB, cingular-angulum bundle; CCG, cingulum–cingulate gyrus bundle; CST, corticospinal tract; ILF, inferior longitudinal fasciculus; SLFp, superior longitudinal fasciculus–parietal terminations; SLFt, superior longitudinal fasciculus–temporal terminations; UF, uncinate fasciculus
Supplementary Table 5. Correlation between serum levels of C4BPA and C4BPB and radial diffusivity values in patients with MDD

| WM tracts   | C4BPA  |     |     | C4BPB  |     |     |
|------------|--------|-----|-----|--------|-----|-----|
|            | r      | p   |     | r      | p   |     |
| Forceps major | -0.154 | 0.335 |     | -0.118 | 0.463 |     |
| Forceps minor | -0.134 | 0.404 |     | -0.145 | 0.366 |     |
| L ATR      | -0.258 | 0.103 |     | -0.238 | 0.133 |     |
| L C4BPA    | -0.148 | 0.356 |     | -0.135 | 0.400 |     |
| L C4BPB    | -0.371 | 0.017* |     | -0.369 | 0.018* |     |
| L CST      | -0.274 | 0.082 |     | -0.264 | 0.095 |     |
| L ILF      | -0.193 | 0.227 |     | -0.170 | 0.289 |     |
| L SLFp     | -0.181 | 0.257 |     | -0.155 | 0.332 |     |
| L SLFt     | -0.237 | 0.135 |     | -0.218 | 0.171 |     |
| L UF       | -0.220 | 0.167 |     | -0.232 | 0.144 |     |
| R ATR      | -0.173 | 0.279 |     | -0.167 | 0.298 |     |
| R C4BPA    | -0.111 | 0.491 |     | -0.122 | 0.449 |     |
| R C4BPB    | -0.492 | 0.001* |     | -0.511 | 0.001* |     |
| R CST      | -0.320 | 0.041 |     | -0.350 | 0.025 |     |
| R ILF      | -0.134 | 0.403 |     | -0.144 | 0.370 |     |
| R SLFp     | -0.278 | 0.079 |     | -0.247 | 0.119 |     |
| R SLFt     | -0.382 | 0.014* |     | -0.359 | 0.021* |     |
| R UF       | -0.120 | 0.454 |     | -0.155 | 0.332 |     |

The r and p-value were obtained using Pearson's correlation analysis including covariates for age and sex. The Bonferroni correction was applied in 18 WM tracts: 18 comparisons in both hemispheres, p<0.00278 (0.05/18). * denotes WM tracts that remained significant after Bonferroni correction. MDD, major depressive disorder; C4BPA, C4b-binding protein alpha chain; C4BPB, C4b-binding protein beta chain; L, left hemisphere; R, right hemisphere; ATR, anterior thalamic radiation; CAB, cingular-angulum bundle; CCG, cingulum–cingulate gyrus bundle; CST, corticospinal tract; ILF, inferior longitudinal fasciculus; SLFp, superior longitudinal fasciculus–parietal terminations; SLFt, superior longitudinal fasciculus–temporal terminations; UF, uncinate fasciculus
Supplementary Table 6. Correlation between serum levels of C4B-PA and C4BPB and axial diffusivity values in patients with MDD

| WM tracts      | C4BPA       | C4BPB       |
|----------------|-------------|-------------|
|                | r    | p   | r    | p   |
| Forceps major  | -0.052 | 0.742 | -0.052 | 0.744 |
| Forceps minor  | -0.229 | 0.145 | -0.176 | 0.265 |
| L ATR          | -0.388 | 0.011* | -0.371 | 0.016* |
| L CAB          | -0.244 | 0.120 | -0.199 | 0.206 |
| L CCG          | -0.382 | 0.013* | -0.360 | 0.019* |
| L CST          | -0.133 | 0.402 | -0.091 | 0.566 |
| L ILF          | -0.077 | 0.627 | -0.028 | 0.861 |
| L SLFp         | 0.065 | 0.681 | 0.086 | 0.590 |
| L SLFt         | 0.106 | 0.504 | 0.137 | 0.388 |
| L UF           | -0.196 | 0.213 | -0.114 | 0.472 |
| R ATR          | -0.397 | 0.009 | -0.333 | 0.031 |
| R CAB          | -0.108 | 0.496 | -0.052 | 0.742 |
| R CCG          | 0.076 | 0.633 | 0.070 | 0.661 |
| R CST          | -0.239 | 0.127 | -0.181 | 0.250 |
| R ILF          | 0.019 | 0.904 | 0.044 | 0.781 |
| R SLFp         | -0.187 | 0.237 | -0.111 | 0.484 |
| R SLFt         | -0.006 | 0.969 | 0.068 | 0.669 |
| R UF           | -0.310 | 0.046 | -0.249 | 0.112 |

The r and p-value were obtained using Pearson’s correlation analysis including covariates for age and sex. The Bonferroni correction was applied in 18 WM tracts: 18 comparisons in both hemispheres, p<0.00278 (0.05/18). *denotes WM tracts that remained significant after Bonferroni correction. MDD, major depressive disorder; C4BPA, C4b-binding protein alpha chain; C4BPB, C4b-binding protein beta chain; L, left hemisphere; R, right hemisphere; ATR, anterior thalamic radiation; CAB, cingular-angulum bundle; CCG, cingulum–cingulate gyrus bundle; CST, corticospinal tract; ILF, inferior longitudinal fasciculus; SLFp, superior longitudinal fasciculus–parietal terminations; SLFt, superior longitudinal fasciculus–temporal terminations; UF, uncinate fasciculus.