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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.
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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).[6-8]

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.[10] In fact,

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many previous studies have shown the role of complement in central nervous system (CNS),[11-13] and alteration of complement components has been suggested to contribute to the pathogenesis of depression by participating in inflammation.[14-16] Moreover, significantly higher levels of C1q, C3, C4, and C5 have been found in patients with MDD than in HC.[14-18] Taken together, these findings imply that complement activation may contribute to the pathogenesis and progression of depression.

C4 binding protein (C4BP), a 570 kDa large plasma glycoprotein composed of seven identical α-chains and one β-chain, is a major soluble inhibitor and second regulator of both the classical and lectin complement pathways.[9,19] In addition to its protective role for the host organism as a circulating complement inhibitor in the innate immune response, C4BP has also been shown to be captured and presented on the surface of human pathogens to evade complement and immune recognition.[20] Furthermore, C4BP colocalizes with Aβ plaques in the brain due to its affinity for serum amyloid P component and CRP, as well as through direct binding to Aβ and dead brain cells.[21,22] These findings indicate that alterations in C4BP levels can reflect the neuroinflammatory state of the host, suggesting that C4BP may become a potential novel biomarker candidate in MDD.

Previous studies in neuroimaging have shown decrease in hippocampus volume and increased level of inflammatory markers such as CRP and IL-6 in patients with MDD.[23-25] Furthermore, increased levels of TNF-α was found to be associated with hyperactivity in the dorsal anterior cingulate cortex and anterior insula, which are areas of the brain known for processing negative affect.[26] In relation to these findings, neuroimaging measures such as structural and functional MRI are effective tools that can be used to investigate whether a heightened 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 regions 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 University 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 severe medical illness, having a comorbid primary neurological illness, currently under pregnancy or nursing, having abnormal results on laboratory finding or physical examination, or contraindicated to MRI were all excluded.

A total of 44 healthy participants were recruited using advertisements for our HC group. Participants who went through structured clinical interviews conducted by two board-certified 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 regarding patient demographics and concurrent psychiatric medications are summarized in Table 1.

The protocol of the present study was approved by the Institutional 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 Declaration 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 vacutainer 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 administered 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 ^{13}C 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/mm^2; 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 study[27] 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 (χ^2=0.053)
Education level			
Elementary and middle school	1	7	
High school or college/university	40	35	0.081 (χ^2=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		
SNRI	5		
Other AD	0		
Combination of ADs	4		
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, p<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		C4BPB	
	r	p	r	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-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. 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 terminations; SLFt, superior longitudinal fasciculus–temporal terminations; 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 SLFt (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,[30] posttraumatic stress disorder,[31] and insomnia.[32] The CAB directly connects the posterior cingulate cortex and subiculum of the hippocampus,[33,34] both of which are part of the default mode network, which is altered in MDD.[35-38] Furthermore, the integrity of the CAB is associated with decision-making, episodic memory, and executive control.[39,40] Which, when disrupted, can cause depressive symptoms. Many studies have suggested a temporal relationship between hippocampal volume and duration of MDD.[41-45] 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 C4BPB levels were higher in patients with MDD. C4BP is known for its role in mediating the cleavage of C3b,[46] a key component necessary for myelin phagocytosis by microglia/macrophages in the CNS.[47,48] 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.[49]

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.[50] This was interesting because most DTI studies have reported decreased FA values in the cingulum region of MDD patients.[51,52] Unlike other WM tracts, previous studies have revealed prolonged cingulum maturation from adolescence up to the mid-20s or later,[53,54] and the peak FA was found to be reached at a mean age of 42 years.[54] 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,[55-57] or it may be the complex result of various physiological factors such as reduced axon diameter, decreased WM branching, and lower intra-voxel crossing.[58] 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.[59]

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, Hyosub Chu, Byung-Joo Ham. Formal analysis: Jihoon Park, Youbin Kang, Un-Beom Kang, Hyosub 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, Hyosub 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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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, corticospinal 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 CAB	-0.148	0.356	-0.135	0.400
L CCG	-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 CAB	-0.111	0.491	-0.122	0.449
R CCG	-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 C4BPA 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