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White matter free water and depressive symptoms in medication-free depressed adolescents: moderation by peripheral inflammation

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Major Depressive Disorder (MDD) often emerges during adolescence and significantly impacts psychological and social functioning. Increasing evidence links both peripheral inflammation and white matter abnormalities to the pathophysiology of MDD. Free-water (FW) imaging, sensitive to neuroinflammatory and microstructural changes, enables investigation of their interplay in depression. However, the role of FW imaging in adolescents with MDD, along with its clinical and inflammatory associations, remains underexplored. Here, we conducted a cross-sectional analysis and exploratory analysis of the relationship between white matter FW, peripheral inflammation, and depressive symptoms in adolescents. 3-T multi-shell diffusion-weighted magnetic resonance imaging data and peripheral cytokine were collected from 147 participants aged 12–18 years, including 63 medication-free adolescents with MDD and 84 healthy controls (HC). FW maps were generated using the DIPY toolbox, followed by voxel-wise analyses conducted with Tract-Based Spatial Statistics in FSL. Our findings reveal that adolescents with MDD exhibited lower levels of inflammatory cytokines, including IFN-γ, IL-2, TNF-α, and IL-4 (all $p < 0.05$), along with significantly reduced white matter FW (family-wise error-corrected $p < 0.05$). Importantly, IFN-γ levels significantly moderated the relationship between altered white matter FW and depressive symptoms ($\beta = 0.46$, $p = 0.003$). Specifically, in adolescents with MDD and higher IFN-γ levels, greater white matter FW was associated with more severe depressive symptoms, while in those with lower IFN-γ levels, higher FW was linked to less severe symptoms. These results suggest that peripheral inflammation, particularly IFN-γ, may be associated with the relationship between white matter FW and the severity of depressive symptoms. This highlights the importance of considering an individual's inflammatory status when interpreting the biological and psychological functioning of adolescents with MDD.

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INTRODUCTION

Major Depressive Disorder (MDD) often manifests during adolescence and early adulthood [1]. Early-onset MDD is associated with a higher risk of recurrent depressive episodes and negative impacts on educational and occupational functioning [2]. Despite available treatment options such as psychotherapy and pharmacotherapy, response rates in adolescents remain limited [3]. Therefore, gaining a better understanding of the underlying pathophysiological mechanisms of MDD in adolescents is crucial for improving treatment efficacy and identifying patients who are most likely to benefit from interventions targeting these markers.

Free-water (FW) imaging is an in vivo diffusion-weighted magnetic resonance imaging (dMRI) technique that builds upon the commonly used diffusion tensor imaging (DTI) method, enabling the quantification of freely diffusing water molecules primarily found in extracellular compartments [4]. While traditional DTI studies in MDD consistently report reduced fractional anisotropy (FA) and increased diffusivity in frontal-limbic circuits, these metrics are confounded by extracellular water, limiting neurobiological interpretation [5]. FW imaging addresses this limitation by separately quantifying extracellular water content and providing tissue-corrected metrics (e.g., tissue-specific fractional anisotropy, FAt) [6]. Elevated FW may indicate neuroinflammatory processes (microglial activation, astrocytic swelling, blood-brain barrier disruption), while tissue-corrected metrics reveal microstructural changes independent of inflammation [7].

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Abnormal FW in the brain white matter has been reported in adults with MDD [8, 9], with FW values are closely associated with depressive symptoms [10]. This technique thus provides complementary insights into inflammation-specific mechanisms that may underlie heterogeneous DTI findings in MDD. To the best of our knowledge, the presence of significant free water alterations in adolescents with MDD has not yet been explored.

Furthermore, there is a lack of research assessing individual difference factors, particularly peripheral inflammation, which could modulate the effects of white matter FW on depressive symptoms. While some studies have reported a significant relationship between white matter FW and depressive symptoms [10, 11], others have shown inconsistent results [12], suggesting that this relationship may be influenced by underlying factors that are not yet fully understood. Both animal and human studies have demonstrated that immune cells produce cytokines, which circulate in the blood and signal the brain through multiple pathways including vagal sensory neurons, active transport across the blood-brain barrier, and passive diffusion in regions with incomplete barrier integrity [13, 14]. Human studies have found that higher peripheral Interleukin-6 (IL-6) levels in adolescents with MDD are associated with reduced white matter fiber connectivity [6]. Additionally, a higher peripheral IL-8/IL-10 ratio is linked to increased white matter FW [12]. Peripheral cytokines are associated with depressive symptoms and the efficacy of antidepressant treatments in adolescents with MDD [15]. Given that peripheral cytokines are linked to both depressive symptoms and the efficacy of antidepressant treatments, it is possible that inflammatory responses in adolescents with MDD may modulate the relationship between white matter FW and depressive symptoms in adolescents, influencing the underlying neurobiological mechanisms of the disorder.

In the current study, we examined the associations among white matter FW, peripheral inflammation, and depressive symptoms in adolescents with MDD, testing whether inflammatory cytokines might play a moderating role in the relationship between altered white matter FW and depressive symptoms. To investigate this, we collected 3-T multi-shell dMRI data and measured 10 pro-/anti-inflammatory peripheral cytokine levels in medication-free adolescents with MDD and healthy controls. Our hypothesis was that inflammatory cytokines would play a moderating role in the relationship between altered white matter FW and depressive symptoms in medication-free adolescents with MDD.

METHODS

Participants

A total of 84 HC and 63 patients diagnosed with MDD were recruited for the cross-sectional of this study. The ethics committees of the Affiliated Brain Hospital of Guangzhou Medical University approved this study (ethical approval number: 2020055), and the written informed consent was obtained from all participants and their parents or legal guardians.

Both MDD patients and HC underwent a structured clinical interview based on the diagnostic and statistical manual of mental disorders, fifth edition. MDD patients met specific inclusion criteria, including being between the ages of 12 and 18, scoring ≥ 17 on the 17-item Hamilton Rating Scale for Depression (HAMD), and being medication-free for at least 4 weeks. All MDD patients were recruited at the outpatient department of the Affiliated Brain Hospital of Guangzhou Medical University. Age and education level-matched HC were recruited through targeted advertising efforts from local community. Exclusion criteria for all participants were a history of developmental disorders, tic disorders, or attention deficit hyperactivity disorder, any other mental illness (e.g., bipolar disorder, posttraumatic stress disorder, and schizophrenia), neurological disorders or other major physical illnesses, immune-inflammatory disorders, and contraindications to MRI scanning. For HC, additional exclusion criteria comprised any history or current diagnosis of MDD or other psychiatric conditions.

The HAMD is divided into five symptom dimensions. The Anxiety/Somatization dimension includes six items related to mental and physical anxiety, gastrointestinal symptoms, general symptoms, hypochondriasis, and self-awareness (Items 10, 11, 12, 13, 15, 17). The Retardation dimension consists of four items addressing depressed mood, work and interest, psychomotor retardation, and sexual symptoms (Items 1, 7, 8, 14). The Cognitive Disturbance dimension includes three items related to feelings of guilt, suicidal thoughts, and agitation (Items 2, 3, 9). The Sleep Disruption dimension assesses difficulty falling asleep, shallow sleep, and early awakening (Items 4, 5, 6). Finally, the Weight dimension evaluates weight loss with a single item (Item 16). Anxiety symptoms were assessed using the Hamilton Anxiety Rating Scale (HAMA) scale.

Cytokine measurement

Upon enrollment in the study, whole blood samples were collected from the participants. All blood samples were collected after a minimum 4-hour fasting period, which was consistent with previous study [16]. Fasting status was confirmed with each participant. Plasma was separated within 30 min of collection using standard centrifugation protocols (3000 rpm for 10 min at 4 °C) and immediately stored at −80 °C until analysis. Plasma was then separated, aliquoted into Eppendorf tubes, and stored at −80 °C for further analysis. The levels of ten peripheral cytokines (interferon gamma [IFN-γ], IL-10, IL-1b, IL-2, IL-4, IL-6, IL-8, Tumor Necrosis Factor-alpha [TNF-α], C-reactive protein [CRP], and Complement component 4 [C4]) were detected by the human high sensitivity T cell magnetic bead panel (Millipore, Billerica, MA, USA, HSTCMAG-28SK) and human neurodegenerative disease magnetic bead panel 2 (Millipore, HNDG2MAG-36K) with the Luminex Magpix-based assay (Luminex corporation), following the manufacturer's instructions. Data generated from the assay were evaluated against a cubic curve fitting and corrected for background readings using Millipore Analyst 5.1 Software (EMD Millipore, Billerica, MA) [17]. In order to achieve normality for statistical analysis, natural log-transformation was applied to all peripheral cytokine values. The intra- and inter-assay coefficients of variation were below 10 and 15%, respectively.

Diffusion MRI acquisition

Participants were scanned using a Siemens Magnetom Prisma 3.0 T MRI Scanner, equipped with a 64-channel head coil, at the Magnetic Resonance Center of Affiliated Brain Hospital of Guangzhou Medical University. Multi-shell dMRI was acquired in the posterior to anterior direction with 64 gradient directions at $b = 1000\ s/mm^2$, and $2000\ s/mm^2$, and 10 interleaved $b = 0\ s/mm^2$ images. An additional 64 gradient directions at $b = 3000$ s/mm2 were collected for tractography studies. However, this $b = 3000$ shell was excluded from all free water modeling calculations to circumvent non-Gaussian diffusion effects that could bias the two-compartment model estimation. To correct for susceptibility-induced distortions, 10 images (b0) with $b = 0\ s/mm^2$ were collected with anterior to posterior phase encoding.

The diffusion sequence was acquired with the following settings: repetition time = 2500 ms, echo time = 83 ms, field of view = 220 mm^2, slice thickness = 2 mm, voxel size = $2 \times 2 \times 2mm^3$.

White matter image processing

During scanning, all dMRI images were visually screened by a trained technician (L.S.) to identify any abnormal radiological or structural features. The Statistical Parametric Mapping software (http://www.fil.ion.ucl.ac.uk/spm) was employed to register all dMRI images to their first b0 images for further validation. No participants were excluded from subsequent analysis based on these screenings. For the dMRI data, image intensities were initially normalized using the mean b0 image. The b0-inhomogeneity distortion was corrected using two opposite phase-encoded images and the "topup" tool in FSL [18]. Additionally, the "eddy" tool in using FSL 6.0.6.5 was employed to correct eddy-current induced field inhomogeneities and head motion for each image volume in a single resampling step [19].

Free-water modeling and tensor estimation were performed using DIPY (version 1.10.0) (http://nipy.org/dipy/index.html), with the two-compartment free-water model fitted to preprocessed diffusion data using dipy.reconst.fwdti. FreeWaterTensorModel with default parameters. In each voxel, the signal was fitted to a two-compartment model, consisting of a FW compartment (isotropic tensor) and a tissue compartment (FW-corrected tensor) [4]. The FW measure represents the relative contribution of FW in each voxel, ranging from 0–1. The tensor of the tissue compartment reflects the tissue microstructure after removing the signal contributed by FW. This study evaluated both the FW component and FAt. Notably, unlike most previous studies, the model was estimated from multi-shell diffusion imaging data, which offers

enhanced stability and robustness compared to single-shell data commonly used in dMRI studies [20].

Voxel-wise analysis was performed using an automated Tract-Based Spatial Statistics (TBSS) pipeline [21]. Initially, individual fractional anisotropy (FA) maps were nonlinearly aligned to a standard space using a target image, which was selected as the most representative FA image (designated with the flag '-n'). This option is recommended for studies involving adolescents and young children. The chosen target image belonged to a 15-year-old participant from the MDD group. Following image registration, a cross-subject mean FA image was generated, which guided the creation of the white matter (WM) tract "skeleton". The threshold for the skeleton was set at FA > 0.2 to include major WM pathways while excluding peripheral tracts that are more susceptible to partial volume effects and inter-subject variability. Subsequently, each subject's FW and FAt data were projected onto the group skeleton for voxel-wise analysis.

For statistical analysis, the randomize function within FSL was utilized to conduct permutation-based nonparametric statistics with 5000 permutations [22]. Significant differences were identified using a p value image, where $p < 0.01$, corrected for multiple comparisons across space through threshold-free cluster enhancement (TFCE) [23]. Anatomical locations were identified using the ICBM-DTI-81 atlas [24].

Statistical analysis

Demographics. Continuous variables were expressed as the mean and standard deviation (SD) or the median and interquartile range (IQR), while categorical variables were presented as frequencies and proportions. Demographic and clinical variables were analyzed using independent t-tests, the Mann-Whitney U-test, and Chi-square tests, respectively. The normality of the data was assessed using the Shapiro-Wilk test. Statistical significance was defined as $p < 0.05$.

Between-group difference in cytokines. A general linear model (GLM) was employed to examine the main effect of diagnosis (HC or MDD) on individual variation in peripheral cytokine levels, while controlling for age and sex as confounding factors in Model 1. Model 2 was additionally adjusted for smoking status (yes or no), drinking status (yes or no), somatic comorbidities (with or without), and family history (yes or no) to assess stability based on Model 1. Each of the ten peripheral cytokines was tested independently, and Bonferroni correction was applied to account for multiple testing.

Between-group differences in white matter microstructure. Between-group comparisons of white matter dMRI parameters, specifically FW and FAt, were conducted using Randomise in FSL. A voxel-wise GLM analysis was performed to assess the equality in FW and FA between the MDD and HC groups while accounting for age and sex as covariates. TFCE was applied for multiple comparison corrections across all skeleton voxels.

Association between cytokines on white matter microstructure in the MDD. Partial correlation was used to test for associations between white matter microstructure (mean FW values extracted from voxels showing significant between-group differences) and depressive symptoms (HAMD scores). We averaged FW values across significant voxels based on: (1) the global nature of systemic inflammation effects on brain tissue; and (2) increased statistical power while reducing multiple comparison burden. Then, we created general linear models that tested the main effects of cytokine levels and white matter microstructure and their interaction. Significant interactions were further analyzed using simple slopes [25], and the Johnson-Neyman method was employed to identify the significant region of moderation [26].

Supplementary analyses. We repeated all regression analyses controlling for additional confounding variables, and removing outliers to test whether the result was robust. Given the small proportion of male patients in our dataset (9 males, 9.5%), we performed the analysis excluding them. We also use HAMA scores, instead of HAMD scores, as outcome variable to test the symptom specificity.

RESULTS

Demographics

Table 1 displays the demographic and clinical information for participants. Compared with HC, MDD patients comprised a significantly lower proportion of males ($\chi^2 = 35.17$, $p < 0.001$) and lower HAMD-17 scores ($Z = 10.53$, $p < 0.001$). No significant differences were observed in age, handedness, smoking, and education between the MDD patients and HC.

Dysregulated serum cytokine in MDD patients

Figure 1 illustrates the peripheral cytokine level comparisons between the MDD patient ($n = 63$) and HC ($n = 84$). After adjusting for sex and age, MDD participants exhibited significantly lower levels of IFN-γ ($F = 14.59$, $p < 0.001$, Cohen's $d = 0.61$), IL-2 ($F = 9.75$, $p = 0.007$, Cohen's $d = 0.68$), IL-4 ($F = 8.22$, $p = 0.012$, Cohen's $d = 0.80$), and TNF-α ($F = 19.40$, $p < 0.001$, Cohen's $d = 1.13$) compared to HC. Furthermore, concentrations of IFN-γ, IL-2, IL-4, and TNF-α remained significantly lower in MDD patients compared with HC after controlling for smoking status, drinking status, somatic comorbidities, and family history (Supplementary Tables 1).

Table 1. Demographic and clinical characteristics of study participants.

Characteristic	HC (N = 84)	MDD (N = 63)	Statistics $t/\chi^2/z$	*P*-value
Age, Years, Median (IQR)	15 (13–16)	14 (13–16)	1.08	0.274
Sex, Male, n (%)	53 (63.1%)	9 (14.3%)	35.17	**<0.001**
Handedness, Right, n (%)	84 (100%)	62 (98.4%)	1.34	0.247
Smoking, Smoker, n (%)	1 (1.2%)	4 (6.4%)	2.91	0.088
Drinking, Yes, n (%)	1 (1.2%)	4 (6.4%)	2.91	0.088
Education, Years, Median (IQR)	9 (7–10)	8 (7–10)	1.06	0.281
Somatic comorbidities, Yes, n (%)	-	10 (15.9%)	-	-
Family history, Yes, n (%)	-	10 (15.9%)	-	-
HAMA, Median (IQR)	0 (0–1)	25 (22–31)	10.52	**<0.001**
HAMD, Median (IQR)	0 (0–2)	24 (21–27)	10.53	**<0.001**
Anxiety/Somatic, Median (IQR)	0 (0–0)	8 (7–9)	10.85	**<0.001**
Retardation, Median (IQR)	0 (0–0)	7 (5–8)	10.16	**<0.001**
Cognitive disturbance, Median (IQR)	0 (0–0)	6 (5–7)	11.08	**<0.001**
Sleep disruption, Median (IQR)	0 (0–0)	4 (2–5)	10.14	**<0.001**
Weight, Median (IQR)	0 (0–0)	0 (0–1)	6.38	**<0.001**

HC healthy controls, *MDD* major depressive disorder, *HAMA* hamilton anxiety rating scale, *HAMD*, 17-item Hamilton Depression Rating Scale.

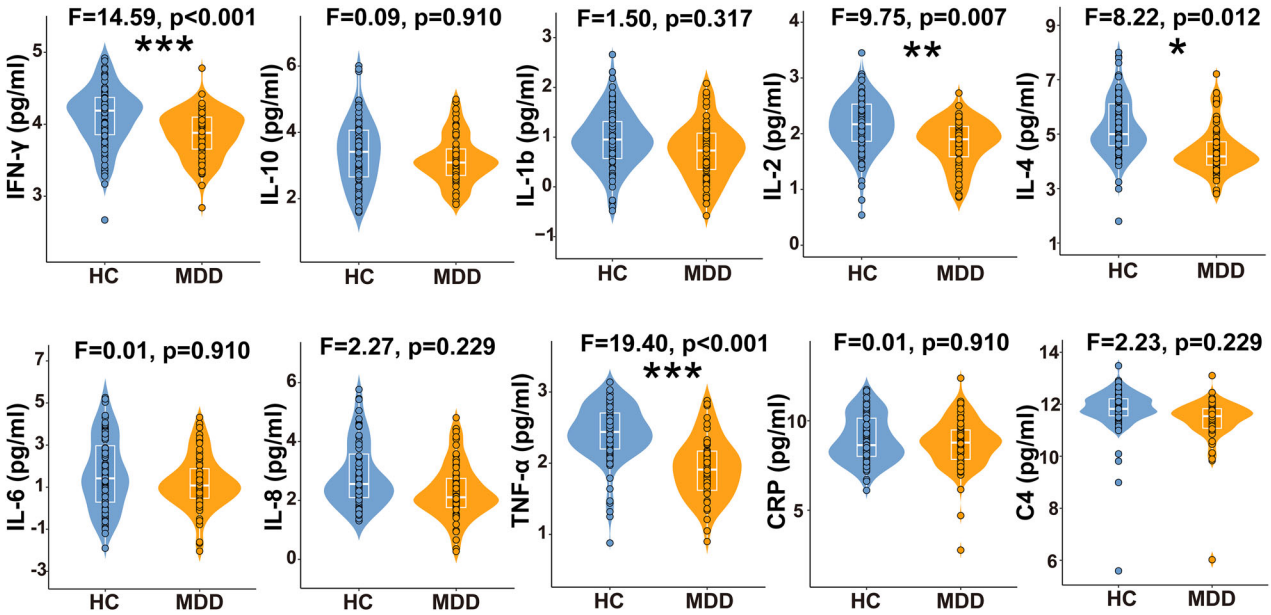

Fig. 1 Betwesen-group comparisons of peripheral cytokine levels with Bonferroni correction, and the analyses were adjusted for age and sex. * p < 0.05; ** p < 0.01; *** p < 0.001. IFN-γ interferon-gamma, IL-2 interleukin-2, IL-4 interleukin-4, TNF-α, tumor necrosis factor-alpha, CRP C-reactive protein, C4 complement component 4, HC healthy control, MDD major depressive disorder.

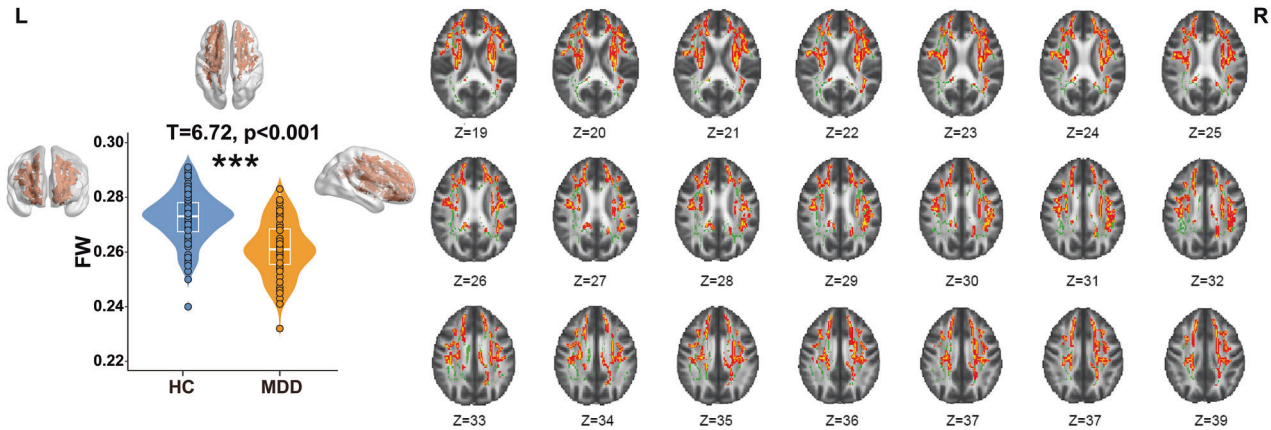

Fig. 2 Between-group comparisons in white matter free-water (FW). Voxel-wise analyses revealed significantly lower FW in major depressive disorder (MDD) patients relative to healthy controls (HC) in tract-based spatial statistics. All voxel-wise analyses controlled for age and sex. * p < 0.05; ** p < 0.01; *** p < 0.001. FW free-water.

Altered FW in MDD

Figure 2 depicts the results of MDD and HC comparisons of FW in voxel-wise TBSS analyses, controlling for age and sex. However, no voxels survived correction for multiple comparisons in FA and FAt, and thus, these two parameters were not further analyzed. The significant clusters were averaged to calculate a single cluster mean for each subject. The results revealed that MDD patients exhibited significantly lower FW values ($t = 6.72$, $p < 0.001$, Cohen's $d = 1.14$). These alterations were widespread, affecting approximately 38.5% of the white matter skeleton (22,835 voxels), including areas such as the genu of corpus callosum, anterior limb of the internal capsule, posterior limb of the internal capsule, anterior corona radiata, superior corona radiata, and superior longitudinal fasciculus.

Association between cytokines on abnormal FW in the MDD

Figure 3 shows the relationships between skeleton-averaged FW and depressive symptoms. Partial correlation analysis revealed a significant positive correlation between FW and HAMD in MDD patients ($r = 0.310$, $p = 0.015$, Fig. 3A), not in HC, after controlling for age and sex. The results remained unchanged after more multivariable adjustments (Supplementary Tables 2).

Among the four differential inflammatory cytokines in MDD and HC, only IFN-γ affected the relationship between FW and HAMD ($F(9, 53) = 2.97$, $R^2 = 0.34$, $p = 0.006$). The main effects of IFN-γ (β[SE] = 0.11 [0.11], $t = -3.07$, $p = 0.003$, Supplementary Tables 3) and of FW (β[SE] = 0.29 [0.11], $t = -2.91$, $p = 0.005$) could predict HAMD score. At the same time, there was a significant interaction of IFN-γ levels and FW when predicting the HAMD score (β[SE] = 0.46 [0.15], $t = 3.09$, $p = 0.003$). Simple slopes analysis indicated that in MDD adolescents with higher IFN-γ level, higher FW was associated with greater severity of depressive symptoms; the direction was reversed in MDD adolescents with lower IFN-γ level. The Johnson-Neyman interval specifying the lower and upper bounds for which IFN-γ significantly moderated the FW-HAMD relation was [3.15, 3.81] (see Fig. 3C&D).

Based on the five symptom dimensions of the HAMD scale, we further explored which symptom dimensions were involved in the modulation of the relationship between FW and inflammatory cytokines. Only IFN-γ significantly moderated the relationship

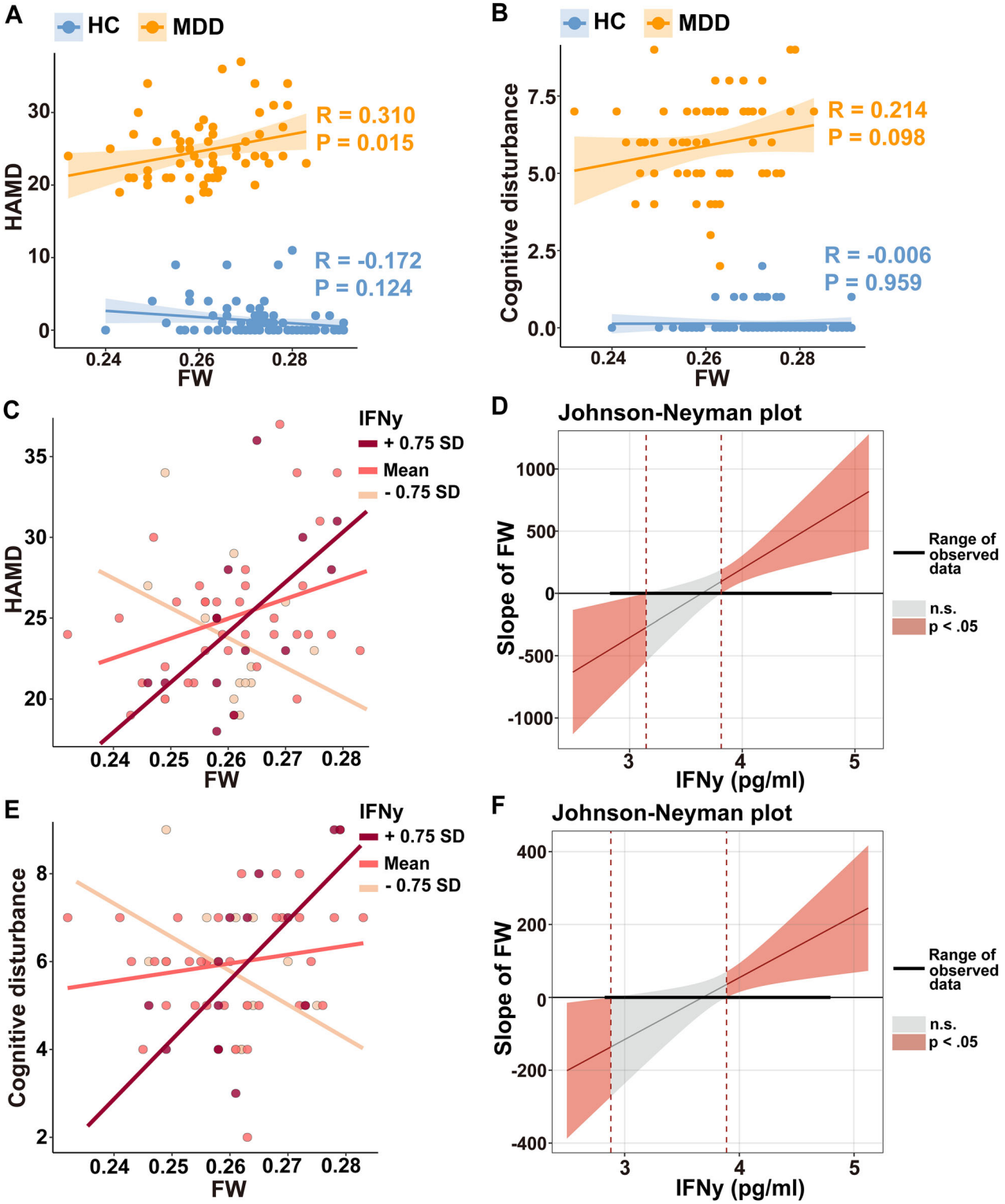

Fig. 3 Association between abnormal free-water (FW) and clinical symptoms. A Partial correlation analysis revealed a significant positive correlation between FW and HAMD score in MDD. **B** Partial correlation showed a positive association between FW and cognitive disturbance in MDD. **C-D** The effect of IFN-γ moderated the FW-HAMD relation. Simple slopes of the IFN-γ-FW interaction (**C**). In MDD who with higher level of IFN-γ (dark red), higher FW was associated with greater severity of depression (higher HAMD scores) whereas in MDD who with lower level of IFN-γ (light red), higher FW was associated with lower severity of depression. Johnson-Neyman plot displaying the level of IFN-γ at which the slope of FW was significant (red) (**D**). Dashed red lines represent the interval boundaries (3.15, 3.81) and the black bar visualizes the observed IFN-γ levels. **E-F** The effect of IFN-γ moderated the association between FW and cognitive disturbance. The meanings of the lines and contours are the same as in Figure C&D. IFN-γ expression was adjusted by natural logarithm. All models were adjusted for sex and age. HAMD hamilton depression scale (17-Items); FW free-water; IFN-γ interferon-gamma.

between FW and cognitive disturbance in the same direction (interaction: β[SE] = 0.41 [0.16], t = 2.54, p = 0.014; see Fig. 3E&F and Supplementary Tables 4). Simple slopes analysis indicated that in MDD adolescents with higher IFN-γ level, higher FW was associated with greater severity cognitive disturbance; the direction was reversed in MDD adolescents with lower IFN-γ level. The Johnson-Neyman interval specifying the lower and upper bounds for which IFN-γ significantly moderated the FW- cognitive disturbance relation was [2.88, 3.89] (see Fig. 3C&D). No other inflammatory cytokines significantly moderated the relationship between FW and the remaining four symptom dimensions.

Further analyses

The sensitivity analyses revealed a similar pattern of significant results (Supplementary Tables 5). Our dataset included a small number of male patients (9 males, 9.5%), and after excluding them, the interaction of IFN-γ levels and FW remained stable in females. Furthermore, after removing outliers, the interaction effect remained significant, indicating that the findings were not influenced by aberrant IFN-γ values. In specificity analyses, when the outcome variable was changed to HAMA scores, the interaction between FW and IFN-γ was no longer significant, suggesting that the observed effect was specific to HAMD (Supplementary Tables 5).

DISCUSSION

To our knowledge, this is the first study to report the associations among white matter FW, peripheral inflammation, and depressive symptoms in adolescents with MDD. We found that adolescents with MDD exhibited lower levels of IFN-γ, IL-2, IL-4, and TNF-α, along with broadly reduced white matter FW. Moreover, we observed that IFN-γ levels significantly moderated the relationship between altered white matter FW and depressive symptoms in adolescents with MDD. Specifically, in adolescents with MDD and higher IFN-γ levels, greater white matter FW was associated with more severe depressive symptoms, while in adolescents with lower IFN-γ levels, higher FW was associated with lower severity of depressive symptoms. These findings provide valuable insights into the underlying pathophysiology of white matter FW in adolescents with MDD, specifically suggesting that IFN-γ levels may regulate this process.

We observed significant differences in the blood levels of four peripheral cytokine components, namely IFN-γ, IL-2, IL-4, and TNF-α, providing further evidence of immune dysregulation in adolescents with MDD, involving both anti-inflammatory and pro-inflammatory processes. Importantly, it is worth noting that the patients in this study were medication-free, and we controlled for potential confounding factors such as smoking and alcohol consumption. Our findings are consistent with previous research, which reported lower levels of IFN-γ [27], IL-2 [27, 28], IL-4 [29], and TNF-α [27] in adolescents with MDD compared to HC. Depression characterized by typical neurovegetative symptoms is associated with hyperactivity of the hypothalamic-pituitary-adrenal axis, leading to elevated cortisol levels (hypercortisolism) [30]. Increased corticotropin-releasing hormone levels contribute to enhanced noradrenergic activity [31], while elevated cortisol levels downregulate the functioning of serotonin 5-hydroxytryptamine type 1 A receptors, resulting in decreased serotonin availability [32]. These changes in neurotransmitter systems, particularly increased noradrenergic signaling and decreased serotonin activity, may contribute to a decrease in the production of Th1 cytokines such as IL-2 and IFN-γ [33].

Our findings related to FA are consistent with previous studies in large sample sizes, which reported no differences in FA when comparing adolescents with MDD to HC [34]. Importantly, in our study, we corrected for partial volume effects using extracellular water to improve the specificity of FA measurements, and we still found no significant differences in FAt between the MDD patients and HC. Previous research has suggested that widespread abnormal FA in adult MDD is primarily driven by recurrent MDD episodes [35]. Therefore, we hypothesize that the lack of significant differences in white matter FAt between MDD patients and HC may be related to the relatively shorter duration and lower number of depressive episodes in adolescent patients.

This study is the first to investigate the abnormalities in white matter FW in adolescents with MDD, while similar research on adult patients is relatively limited. However, in contrast to our findings, previous studies have reported a significant increase in white matter FW in adults with MDD [12]. Interestingly, an increase in white matter FW has also been observed in adults with schizophrenia [36]. Importantly, white matter FW levels were closely related to age. A study including healthy individuals aged 25–94 showed that white matter FW followed a U-shaped curve with age, rapidly declining in early stages and subsequently increasing after reaching the trough [37]. This suggests that, during adolescence, increases in age are associated with decreases in FW, while in older age, increases in age are associated with increases in FW. Diffusion magnetic resonance studies in non-primate animals have similarly shown a U-shaped trajectory of white matter FW from early childhood to late adulthood [38]. These findings suggest that the trajectory of white matter FW development in individuals with psychiatric disorders may be altered, particularly during adolescence, where early developmental changes may occur. The stress acceleration theory posits that early adverse experiences, characterized by chronic stress, may affect brain development by increasing glucocorticoids [39].

Our study reveals an apparent paradox requiring clarification: while MDD adolescents showed overall reduced white matter FW compared to controls, within the MDD group, the relationship between FW and symptom severity was bidirectionally moderated by IFN-γ levels. The group-level FW reduction likely reflects general pathophysiological processes in adolescent depression, such as increased glial density or altered tissue organization. However, within this reduced range, the clinical significance of FW values depends critically on inflammatory status. In adolescents with higher IFN-γ, relatively higher FW values (though still below normal) correlated with worse symptoms, suggesting active neuroinflammatory processes involving blood-brain barrier disruption or inflammatory infiltration [40]. Conversely, in those with lower IFN-γ, lower FW values were associated with more severe symptoms, potentially reflecting structural alterations without active inflammation [5]. This bidirectional moderation indicates that adolescent MDD comprises heterogeneous subtypes with distinct pathophysiology: an inflammatory subtype where relative FW increases signal active inflammation and worse outcomes, and a non-inflammatory subtype where greater FW reduction indicates structural changes linked to symptom severity. These findings underscore that the same FW value may have opposite clinical implications depending on the patient's inflammatory profile, highlighting the importance of considering individual inflammatory status when interpreting neuroimaging biomarkers in adolescent depression [14].

The bidirectional moderation by IFN-γ reveals that FW's neurobiological significance is fundamentally context-dependent. In high inflammatory states, elevated FW reflects maladaptive processes—blood-brain barrier disruption, microglial activation, and vasogenic edema—driving neuronal dysfunction and symptom exacerbation [41]. Specifically, in the high IFN-γ context, these processes are accompanied by cytokine-induced excitotoxicity, oxidative stress, and disrupted neuron-glia interactions [14]. Conversely, in low inflammatory states, relatively higher FW may indicate preserved homeostatic mechanisms including intact glial support and myelin integrity, potentially serving protective functions. In adolescents with low IFN-γ, higher FW values (though

still below healthy controls) may reflect adaptive neuroplasticity or more normative developmental processes, such as maintained synaptic pruning efficiency, preserved oligodendrocyte function [42], and intact neurotrophic support. This aligns with emerging evidence that FW is a sensitive but non-specific biomarker whose clinical meaning depends on the neuroimmune milieu. IFN-γ functions as a biological "switch" [43] that transforms the pathophysiological meaning of FW alterations--what is adaptive in one context becomes pathological in another. This framework underscores the necessity of integrating inflammatory profiling with neuroimaging to accurately interpret white matter changes in adolescent depression, moving toward a systems-level understanding of depression heterogeneity.

This study has several limitations. (1) Although we controlled for variables such as age, gender, smoking, and alcohol use in our analysis, we did not account for other potential confounding factors, such as body mass index, socioeconomic status, and pubertal stage, which may influence peripheral immune status. Future studies should control for a wider range of confounding variables. (2) FW is visible within the low b-value range, and we did not use high b-value shells in this study, as they are not suitable for inclusion in the FW-DTI Gaussian diffusion model. The information from high b-value shells remains to be explored further in future studies using non-Gaussian models or fiber tracking techniques. Future research could also focus on optimizing the sequence parameters. (3) Given that immunological activation and cytokine responses in depression may exhibit temporal dynamics, it would be valuable for future studies to include longitudinal investigations. Long-term follow-up of patients could provide valuable insights into the alterations of peripheral cytokines and FW throughout the course of depression. (4) Our MDD sample was predominantly female, reflecting the higher prevalence of depression among adolescent females in clinical settings. Considering established sex differences in inflammatory responses and white matter development, the generalizability of our findings to male adolescents with MDD may be limited. Future studies with balanced sex representation are needed to validate these results across sexes.

CONCLUSIONS

Our findings suggest that IFN-γ levels significantly moderated the relationship between altered white matter FW and depressive symptoms in adolescents with MDD. These results underscore the importance of considering an individual's peripheral inflammatory status when interpreting the biological and psychological functioning of adolescents with MDD. Furthermore, our findings provide valuable insights for developing targeted treatment strategies, emphasizing the role of inflammation in adolescents with MDD.

DATA AVAILABILITY

Data is available on request from the authors.

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AUTHOR CONTRIBUTIONS

LW and YZ were responsible for conceptualization, methodology, software, visualization, data curation, and writing (both original draft and review & editing). WC and LX contributed to methodology, resources, and investigation. ZF, HZ, CX, LZ, ZY, CYY, CYF, and MSM performed methodology, resources, and investigation. SR and LH conducted editing and investigation. MSR provided resources. SX helped write the manuscript. NY and ZY contributed to conceptualization, funding acquisition, investigation, supervision, and project administration.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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