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Inflammatory cytokines and callosal white matter microstructure in adolescents

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ABSTRACT

Adolescent depression is characterized by heightened inflammation and altered connectivity of fronto-cingulate-limbic tracts, including the genu of the corpus callosum (CCG) and the uncinate fasciculus (UF). No studies, however, have yet examined the association between inflammation, measured by peripheral levels of cytokines, and white matter connectivity of fronto-cingulate-limbic tracts in adolescents. Here, 56 depressed adolescents (32 females, 3 non-binary; 16.23 ± 1.28 years) and 19 controls (10 females; 15.72 ± 1.17 years) completed a diffusion-weighted MRI scan at 3 Tesla. We conducted deterministic tractography to segment bilateral corpus callosum (genu and splenium) and UF and computed mean fractional anisotropy (FA) in each tract. A subset of participants (43 depressed and 17 healthy controls) also provided dried blood spot samples from which we assayed interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) using a Luminex multiplex array. Depressed participants did not differ from controls in FA of the corpus callosum or UF (all FDR-corrected *p*s > 0.056) but exhibited higher levels of inflammation than did controls (IL-6: $\beta = 0.91$, FDR-corrected $p = 0.006$; TNF-α: $\beta = 0.76$, FDR-corrected $p = 0.006$). Although diagnostic group did not moderate the associations between inflammatory cytokines and FA in the CCG and UF, across both groups, greater peripheral inflammation was associated with lower FA in the CCG (IL-6: $\beta = -0.38$; FDR-corrected $p = 0.044$; TNF-α: $\beta = -0.41$, FDR-corrected $p = 0.044$). This study is the first to examine associations between peripheral inflammation and white matter microstructure of fronto-cingulate-limbic tracts in depressed and nondepressed adolescents. Future mechanistic studies are needed to confirm our findings; nevertheless, our results suggest that heightened inflammation is an important component of neurophenotypes that are relevant to adolescent depression.

1. Introduction

In 2016, 16 million adults and 3 million adolescents in the United States experienced depression (NIMH, 2017), exacting a cost of over $210 billion per year in lost productivity and economic growth (Kuhl, 2018). Due to the recurrent nature of depressive episodes, depression poses a serious public health concern, particularly among adolescents. Indeed, the incidence of depression in adolescents is increasing (Breslau et al., 2017), with recent data indicating that the prevalence has worsened as a result of the COVID-19 pandemic (Racine et al., 2021). Adolescent-onset depression is associated with a more severe and recurrent clinical course of depression, and with a greater risk of suicide (Clark et al., 2007; CDC, 2007; Thapar et al., 2012). Therefore, it is critical to focus research efforts on elucidating neurobiological pathways that may be important targets for effective approaches to intervention and prevention during this vulnerable period (Colman & Ataullahjan, 2010).

Experiencing stressful life events is one of the strongest predictors of the development of depression in adolescents (Andersen & Teicher, 2008; Hammen et al., 2010; Slavich & Irwin, 2014); consequently, researchers have sought to examine the mechanisms by which stress may affect brain development. Such efforts have focused on immune

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responses, given that psychosocial stress has been shown to activate the immune system in a manner similar to infections, even when the perceived stressor presents no immediate threat (Jankord et al., 2010; Slavich & Irwin, 2014). Specifically, psychosocial stress increases production of peripheral proteins, including pro-inflammatory cytokines (e.g., interleukin-6, IL-6; tumor necrosis-alpha, TNF-α), that act as immune mediators and stimulate the hypothalamic-pituitaryadrenal (HPA) axis (Miller et al., 2009). Adolescence is a particularly sensitive developmental period marked by significant changes in heightened hormonal responses that are both modulated by stress exposure and directly affect HPA-axis functioning (Romeo, 2013). Particularly in the context of ongoing adolescence-specific hormonal changes, stress-related immune system activation likely contributes to the prevalence of mental health conditions characterized by maladaptive responses to stress, such as depression, during adolescence (Breslau et al., 2017).

Several studies in depressed and nondepressed humans have demonstrated that stress is associated with higher levels of both pro-inflammatory cytokines and depressive symptoms (Slavich & Irwin, 2014, Slavich et al., 2010; Miller & Raison, 2016). Moreover, there is strong meta-analytic evidence that peripheral levels of IL-6 and TNF-α are consistently higher in depressed patients than in healthy controls (Liu et al., 2012). This association may be bidirectional and reflect a positive feedback loop, as recent meta-analytic data indicate that higher levels of IL-6 predict levels of future depressive symptoms, which, in turn, predict higher levels of IL-6 (Giollabhui et al., 2020). Finally, mechanistic studies also support the role of inflammation as a driver of depressive symptomatology (for a review, see Dooley et al., 2018). For example, Eisenberger et al. (2010) examined the effects of an endotoxin versus placebo on IL-6 and TNF-α concentrations and self-reported social disconnection and depressed mood in 39 healthy young adults. The researchers found that compared to the group that received the placebo, the group that received endotoxin administration had higher concentrations of IL-6 and TNF-α as well as increases in self-reported feelings of social disconnection and depressive symptoms, suggesting the role of inflammation in precipitating depression.

The precise mechanisms by which inflammation affects depression-related brain circuits, however, are not well understood. Peripheral cytokines are able to access the brain through multiple pathways, including through saturable transporters for ILs and TNFs, through fenestrated capillaries in circumventricular organs that active the HPA-axis (for a review, see Quan & Banks, 2007), and through the trafficking of immune cells to the brain that interact with microglia, thereby prompting the release of cytokines and other inflammatory mediators in the central nervous system (for a review, see Haroon et al., 2017). Importantly, increased levels of cytokines in the central nervous system adversely affect the integrity of several glial elements, including oligodendrocytes, that generate myelin and are a dominant cell type for white matter in the brain. Current data also indicate that peripheral immune responses stimulate chemokines produced by microglial cells that promote the trafficking of monocytes and macrophages to specific brain regions, including the anterior cingulate cortex (Torres-Platas et al., 2014) and amygdala (Wohleb et al., 2013), both of which are regions strongly implicated in depression. Collectively, these studies indicate that there are multiple biologically plausible pathways by which peripheral inflammation may affect white matter morphometry and microstructure in fronto-cingulate-limbic regions.

Compared to healthy controls, depressed adults and adolescents exhibit reduced white matter connectivity, as measured by fractional anisotropy (FA, an index of directionality of water diffusion and, thus, of microstructural properties including membrane integrity and myelin thickness; Kochunov et al. 2007). This reduced white matter connectivity is seen in major fronto-cingulate-limbic white matter tracts, such as the corpus callosum and uncinate fasciculus (Bhatia et al, 2018; LeWinn et al., 2014; Van Velzen et al., 2020; Wise et al., 2016). The corpus callosum specifically connects the left and right hemispheres of the brain and, accordingly, supports the integration of emotional, cognitive, linguistic, and perceptual processing (Hinkley et al., 2012). Although several portions of the corpus callosum have been found to be altered in individuals with depression (Van Velzen et al., 2020; Wise et al., 2016), the genu of the corpus callosum (CCG) may have specific relevance for depression, given that the CCG is proximal to the left and right prefrontal and anterior cingulate cortices, and relays affective and cognitive signals from these regions. Indeed, morphological differences in the corpus callosum between depressed and nondepressed adolescents have been found to be driven specifically by differences in the CCG (MacMaster et al., 2013).

Similarly, the uncinate fasciculus (UF) is a white matter tract that connects portions of mesial temporal cortex, including amygdala and hippocampal structures, with ventromedial prefrontal cortex and lateral orbitofrontal cortex. Importantly, the UF is the one of last major tracts to reach adult-like levels of myelination (Olson et al., 2015; Simmonds et al., 2014) and is thought to support inhibition signaling from the prefrontal cortex to temporal lobe and limbic structures (Hornberger et al., 2011; Mincic, 2015). Researchers have found lower FA values in adolescents who have been exposed to higher levels of stress, suggesting that lower FA of the UF may be a risk factor for depression and anxiety (Hanson et al., 2015; Ho et al., 2017). Consistent with this formulation are results from several studies of pediatric depression and anxiety, which have documented lower FA in those with these conditions compared to healthy controls (LeWinn et al., 2014; Tromp et al., 2019; although see Aghajani et al., 2014; Ho et al., 2021a for contrary results).

The studies reviewed above highlight the roles of elevated inflammation and compromised white matter organization of the CCG and UF in adolescents with depression. No study, however, has directly examined associations between peripheral levels of inflammation and measures of fronto-cingulate-limbic white matter microstructure in human adolescents. To this end, we assessed peripheral inflammatory cytokines and white matter microstructure of the CCG, the corpus callosum splenium (CCS), and the UF in 60 adolescents (43 depressed and 17 psychiatrically healthy controls) and tested whether higher levels of inflammatory cytokines were associated with lower FA in white matter tracts and whether these associations depended on depression diagnosis.

2. Methods

2.1. Participants

Participants were recruited from the Teen Inflammation Glutamate Emotion Research (TIGER) study (Walker et al., 2020), an ongoing longitudinal neuroimaging study of adolescent depression. Potentially depressed adolescent participants were recruited from the San Francisco Bay Area using social media and online advertisements. To be included in the study, participants had to be between the ages of 13 and 18 years, English speakers, meet diagnostic criteria for threshold or subthreshold MDD or dysthymia, and have a Children's Depression Rating Scale-Revised (CDRS-R) total t-score $\geq$ 55 (see *Clinical Assessments* for more details). Exclusion criteria included history of neurological disorder or major medical illness, any cognitive or physical challenges that would limit adolescents' ability to understand or complete study procedures, contraindications for MRI (e.g., had metal implants, braces, claustrophobia), meeting criteria for current or lifetime psychosis/mania or current substance dependence, and symptoms of concussion within the past 6 weeks or loss of consciousness during any lifetime concussion. Psychiatrically healthy control (CTL) participants were recruited from the community using similar methods. Inclusion and exclusion criteria for CTL participants were the same as those in the MDD group, with the exception that CTL participants could not meet current or lifetime diagnostic criteria for any Axis I disorder and could not have a first-degree relative with a history of suicide or definite or suspected history of depression, bipolar disorder, or schizophrenia. This study was approved by the Institutional Review Boards at Stanford University and the University of California, San Francisco. In accordance with the

Declaration of Helsinki, all participants provided informed assent and their parent/legal guardian provided informed consent and were financially compensated for their participation in these studies.

Of the 75 participants (56 MDD, 19 CTL) who met eligibility criteria and provided usable scan data (structural MRI and diffusion MRI), 15 participants (13 MDD, 2 CTL) did not provide blood samples for cytokine analysis. There were statistically significant differences between the 60 participants who were included in the present analyses and the 15 who were not in diagnostic group status ($\chi(1)^2 = 11.267$; $p < 0.001$), gender identity ($\chi(2)^2 = 24.699$; $p < 0.00001$), race ($\chi(5)^2 = 54$; $p < 0.001$), ethnicity ($\chi(1)^2 = 29.4$; $p < 0.001$), and parental education levels ($\chi(5)^2 = 49.586$; $p < 0.0001$): those without full usable data were more likely to have MDD, to identify as female, white, or Non-Hispanic, and to have parents with lower educational attainment. Importantly, participants who were ultimately included in the present analysis did not differ from those who were not included on age, biological sex, depression severity scores (RADS-2), FA values, and motion during the scan (all $ps > 0.213$).

2.2. Neuroimaging acquisition

All participants except for six (all MDD) were scanned on a 3 T Discovery MR750 (GE Medical Systems, Milwaukee, WI) with a 32-channel head coil (Nova Medical) at the Center for Cognitive Neuroscience and Neurobiological Imaging located in the Department of Psychology at Stanford University. The remaining six were assessed after a scanner hardware upgrade to a 3 T SIGNA Ultra High Performance that coincided with when COVID-19 mitigation procedures were put in place; thus, in all statistical analyses we also included scan timepoint (pre-COVID, post-COVID) as a binary covariate. While the pre- and post-COVID MDD participants did not differ in RADS-2 (pre-COVID mean = 80.8, post-COVID mean = 76.5, $p = 0.386$) or IL-6 (pre-COVID mean = 4.85, post-COVID = 5.23, $p = 0.272$), the post-COVID group did show significantly higher levels of TNF-α (pre-COVID = 4.59, post-COVID = 4.73, $p = 0.014$). A high-resolution T1-weighted anatomical scan was acquired using an SPGR sequence (TR/TE/TI = 8.2/3.2/600 ms; flip angle = 12°; 156 axial slices; 1.0 mm isotropic voxels). All participants completed a diffusion-weighted scan using an EPI sequence (TR/TE = 8500/93.5 ms; 64 axial slices; 2 mm isotropic voxels; 60 b = 2000 diffusion-weighted directions, and 6 b = 0 acquisitions at the beginning of the scan; A/P phase encoding direction). At the end of each scan, participant height and weight were collected to calculate body mass index (BMI), which was used as a covariate in our statistical analyses.

2.3. Deterministic tractography using automated fiber quantification (AFQ)

Diffusion MRI data were processed using the open source mrVista software distribution developed by the VISTA lab (https://vistalab.stanford.edu/). Whole-brain fiber tracts were first mapped onto an anterior-posterior commissure-aligned T1-weighted image (Yeatman et al., 2012; Ho et al., 2017). As in previous work (Ho et al., 2017; Ho et al., 2021b), we minimized the effects of motion on the data by first excluding directions (i.e., volumes) where relative motion in the translational directions or the rotational directions exceeded 5 mm or 1.5°, respectively. All participants included in the present study did not have more than 12 such outlier volumes (20% of volumes). Streamlines in each of the four tracts of interest—CCG, CCS, left UF, right UF—were automatically generated using a two planar waypoint region of interest (ROI) approach and then seeding these two ROIs in accordance with probabilistic fiber groupings (see Yeatman et al., 2012 for more details). Candidate fibers were then assessed based on similarity to the standard probability map (Wakana et al., 2007; Mori et al, 2002). We excluded outliers, defined as exceeding 4 standard deviations from the spatial core of the tract, until no outlier volumes existed in the tract. All tracts were visually inspected for consistency and any tracts that were resolved inadequately or in ways that were biologically implausible were omitted from analyses (Ho et al., 2017; Ho et al., 2021a). As AFQ computes diffusivity metrics for 100 evenly spaced nodes along the tract, we averaged diffusivity metrics along the entire tract for a more reliable estimate (Kircanski et al., 2019; Ho et al., 2021a). See Fig. 1 for tract visualizations from a representative subject.

2.4. Inflammatory markers

All blood samples were collected on the same day as the MRI scan, immediately following the MRI scan. Inflammatory cytokines were assayed from dried blood spots using a protocol that has had over a 95% successful compliance rate in youth (Walker et al., 2020; Ho et al., 2021a). In this protocol, 4–5 blood spots, each approximately 150–250 μL per spot, were collected using mini contact-activated lancets (BD 366,594 Microtainer, BD Biosciences, San Jose, CA) to prick the finger after the participants held their non-dominant hand under hot water for 2 min. With the participants' consent, we asked that they complete 10 jumping jacks to increase blood flow prior to blood sampling. Blood spots were collected on 0.5 in. filter paper cards (Whatman #903, GE Healthcare, Pascataway, NJ) and then dried overnight at room temperature before being transferred to Ziplock bags with a desiccant for storage in a −20 °C freezer. All samples were assayed at the Stanford Human Immune Monitoring Center (HIMC). For extraction and analysis, a single dried blood spot was cut out and placed in a 1.5 mL tube containing 400 μL radioimmunoprecipitation buffer (RIPA Boston) and protease inhibitor. This mixture was then incubated overnight at 4 °C with shaking (500–600 rpm). Samples were then centrifuged for 10 min at room temperature at 17,000 rpm and liquid transferred to a clean polypropylene plate. Samples were diluted 3-fold in the Luminex assay buffer prior to running the 62-plex Luminex assays (catalog # EPX620-00000–801 from Affymetrix/eBioscience; San Diego, CA). Samples were added to a 96-well plate that contained the washed antibody-linked magnetic beads before being incubated overnight at 4 °C with shaking (500–600 rpm). Following overnight incubation, the plates were washed again (ELx 405 Biotek washer) and a biotinylated detection antibody was added for 60 min at room temperature with shaking. The plate was then washed and streptavidin-PE was added for 30 min at room temperature with shaking. The assay plate was then washed again and a reading buffer was added to the wells. All samples were run through a dedicated flow cytometry-based platform, the Luminex FlexMap 3D (Luminex Corporation). Samples with a bead count < 50 were not included for analysis (however, no analyte in our analyses met this criterion). The intra-assay (replicates extracted and run in the same plate) coefficients of variation (CV) for IL-6 and TNF-α were 7.80% ± 5.69% and 6.68% ± 4.92%, respectively. The lower limits of detection for IL-6 and TNF-α were 8.14 pg/mL and 5.89 pg/mL, respectively.

In addition to the 62-plex beads, each well also contained Assay Chex (Radix BioSolutions), process control beads that allowed us to normalize the data based on potential confounds (e.g., non-specific binding). Data were analyzed using MasterPlex software (Hitachi Software Engineering America Ltd., MiraiBio Group). Although both median fluorescence intensity (MFI) and calculated concentration values (in pg/mL) were reported for each analyte, based on prior work demonstrating advantages of using MFI over concentration values for low abundant analytes (Breen et al., 2016; Rosenberg-Hasson et al., 2014), we conducted all statistical analyses using MFI values (analyses with pg/mL values are provided in the Supplement; see Tables S2–S4). As in previous work (Ho et al., 2021a), we log transformed all MFI values for each analyte and then computed an estimate of each analyte accounting for technical error using orthogonal nonlinear least squares (R package *onls*). We focused on IL-6 and TNF-α to minimize multiple comparisons and pursue hypothesis-driven analyses based on prior literature relating inflammatory cytokines with depression (Howren et al., 2009; Liu et al., 2012; Raison et al., 2013; Valkanova et al., 2013; Liu et al., 2020). Critically, the cytokines we assayed using the dried blood spot protocols have been shown to be strongly correlated with plasma cytokine concentrations

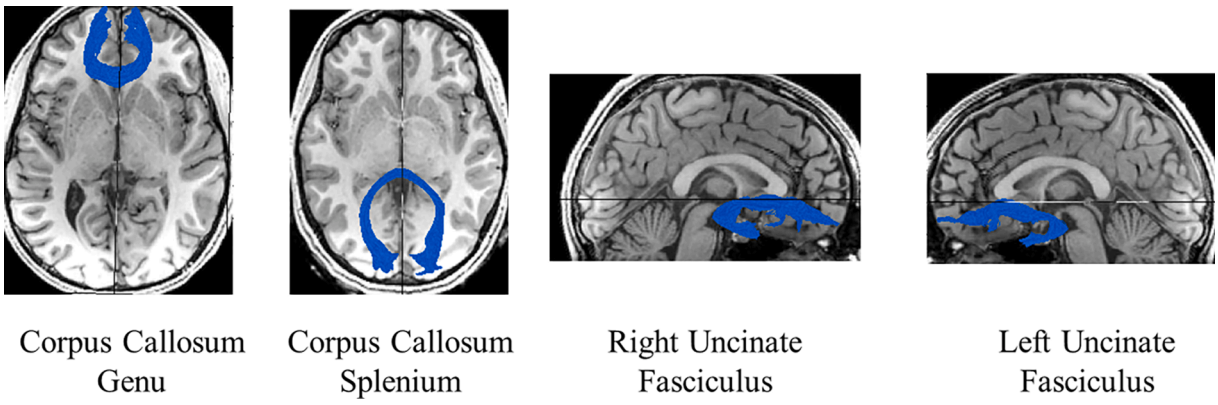

Fig. 1. Visualizations of corpus callosum genu (CCG), splenium (CCS), and bilateral uncinate fasciculus (UF) from a representative subject.

(although see Fig. S1, where we found strong correspondence in IL-6 but not in TNF-α; Rosenberg-Hasson et al., 2020; McDade et al., 2020). It is important to emphasize that the concentration values reported here will be different from those using different vendors and different antibody pairs and different cytokine standards. Several published papers have noted the incongruence among different vendors' kits (Siawaya et al., 2008; Günther et al., 2020). Given frequent lack of concordance between immunoassays from different vendors, it is not too surprising that correlations among analytes would also differ when using one vendor kit versus another. The stronger correlation between IL-6 and TNF-α in our study (see Fig. 2) versus previous work could be due to better sensitivity for one or both of these analytes in our kits. Blood samples were assayed on a single plate at three separate dates: February 2019, October 2019, and June 2021. In all statistical analyses involving cytokines, we controlled for assay batch as a categorical factor.

2.5. Clinical assessments

To make a diagnosis of depression and determine inclusion into the study, we conducted clinical interviews using the Kiddie Schedule for Affective Disorders and Schizophrenia, Present and Lifetime (K-SADS-PL) based on DSM-IV criteria (see Walker et al., 2020 for more details). The KSADS-PL is a semi-structured interview that assesses current and past symptoms of mood, anxiety, psychotic, and disruptive behavior disorders, incorporating child and parent report as well as clinician judgment (Kaufman et al, 1997). All participants also completed the Children's Depression Rating Scale-Revised (CDRS-R), a 17-item rating scale used by clinicians to assess severity of depression and change in depressive symptoms dimensionally in children and adolescents (Poznanski and Mokros, 1996). All depressed participants in the study who were subthreshold for MDD or dysthymia were required to have a *t*-score $\geq$ 55 on the CDRS-R to be included in the study. To assess self-reported severity of depression, all adolescents completed the Reynolds Adolescent Depression Scale (RADS-2), a 30-item scale that assesses depressive symptoms and has been shown to demonstrate excellent test re-test reliability in adolescents (Reynolds, 2002).

2.6. Statistical analyses

All statistical analyses were conducted using R version 4.1.0 for MacOS BigSur, including packages from the following libraries: *corrplot*, *dabestr*, *gvlma*, *p.adjust*, *parameters*, *performance*, *psych*, and *tidyverse*. Chi-square tests were conducted to determine whether the distribution of individuals who identified as each gender (and, separately, sex at birth), and race differed between the two diagnostic groups. Student's *t*-tests were conducted to compare mean scores on key metrics between the two diagnostic groups. Finally, we used linear regression models to examine: 1) group differences in IL-6 and TNF-α; 2) group differences in FA in the corpus callosum and uncinate tracts; 3) associations between each inflammatory cytokine and FA in the four tracts of interest across the entire sample; and 4) the moderating effect of diagnostic group on association between each inflammatory cytokine and the four tracts of interest. In all primary statistical analyses, we included age, BMI (where appropriate), batch (where appropriate), scan timepoint, and tract length (where appropriate) as covariates. All statistical models met assumptions for linear modeling (normality and non-heteroscedasticity of

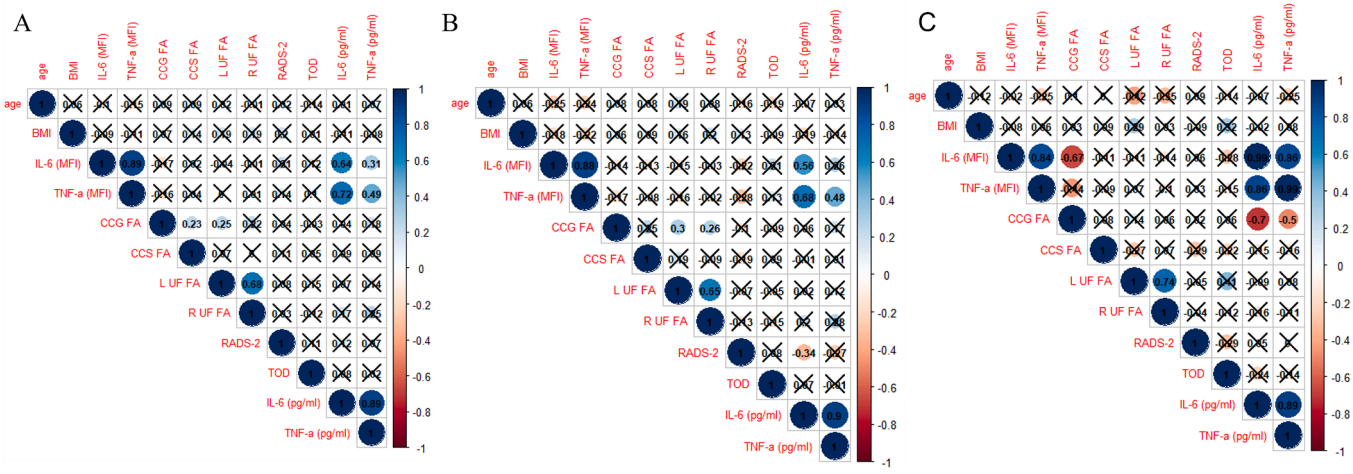

Fig. 2. Correlation matrix summarizing bivariate correlations (Pearson's *r*) among key variables and covariates across all participants (A), in the MDD group only (B), and in the CTL group only (C). Correlations that are significant at $p < 0.05$ (uncorrected) are displayed without an 'X'.

residuals, acceptable skew and kurtosis) using *gvlma* and the Shapiro–Wilk test of normality (applied to the residuals of each model). For each of these primary analyses, we applied FDR-correction for significance; all other *p*-values reported are uncorrected. In subsequent sensitivity analyses for models that yielded either significant group effects or significant effects of inflammatory cytokines on tract FA, we also included psychiatric medication (as a binary factor) and other confounding medications that were for non-psychiatric uses, including corticosteroids, NSAIDs, anti-histamines (as a binary factor), as well as approximate time of day as additional covariates. In post hoc analyses for models that exhibited significant effects of inflammatory cytokines on tract FA, we also tested whether cytokine levels were also associated with other diffusivity metrics of that tract (i.e., axial diffusivity, radial diffusivity, and mean diffusivity). Finally, as an exploratory supplemental analysis, we applied tract-based spatial statistics (TBSS) following procedures described in van Velzen et al. (2020), which is the largest comparison of adult and adolescent MDD versus CTL on diffusivity metrics, and related IL-6 and TNF-α to FA in the 42 white matter tracts that were tested in this paper.

3. Results

3.1. Participant characteristics

Demographic and clinical characteristics of the participants are presented in Table 1. As expected, the adolescents with MDD reported significantly higher self-reported depression severity, as measured by RADS-2 scores, and greater use of psychiatric medications (all *ps* < 0.001). The two diagnostic groups, however, did not differ in any demographic variables, non-psychiatric medication use, tract length in any of the corpus callosum or uncinate fasciculus tracts, or in motion during the diffusion-weighted scan (all *ps* > 0.08). We also present bivariate correlations between all key variables and covariates in Fig. 2. Interestingly, other studies have reported robust associations between IL-6 and BMI (Stumper et al., 2020), which we did not observe in our study sample. For a brief discussion on the absence of an association between IL-6 and BMI, please see the Supplement.

3.2. Group differences in inflammatory cytokines and fractional anisotropy

We tested whether there was a diagnostic group difference in pro-inflammatory cytokine concentrations and in FA of the CCG, CCS, and in left and right UF. When accounting for age, gender, BMI, scan timepoint (post-upgrade/during COVID-19), and assay batch as covariates, we found that the MDD group had significantly higher levels of IL-6 (β = 0.91, 95% CI: [0.32, 1.50], FDR-corrected p = 0.006) and TNF-α (β = 0.76, 95% CI: [0.23, 1.29], FDR-corrected p = 0.006) than did the CTL group. See Fig. 3 for more details. Including time of day as an additional covariate did not change these results (IL-6: uncorrected p = 0.005; TNF-α: uncorrected p = 0.008). When accounting for age, gender, scan timepoint, and tract length as covariates, the groups did not differ in FA of any of the tracts after applying FDR-correction (all *ps* > 0.064). Without correction, the groups did not differ in FA of the CCG or UF (all *ps* > 0.168) but did differ in FA of the CCS (β = 0.68, 95% CI: [0.13, 1.22], p = 0.016): adolescents with MDD exhibited greater FA compared to CTL in this tract. See the Supplement and Table S1 for a summary of the associations between severity of depression, as measured by RADS-2 scores (log transformed), and each of these variables.

3.3. Higher levels of inflammatory cytokines are associated with lower fractional anisotropy in the corpus callosum genu

We tested whether IL-6 and TNF-α were associated with FA in the callosal and uncinate tracts across the entire sample, and whether diagnostic group moderated any of these associations. When accounting for diagnostic group, age, gender, BMI, assay batch, scan timepoint, and tract length, we found that greater concentrations of IL-6 and of TNF-α were each significantly associated with lower FA in the CCG (IL-6: β = −0.38, FDR-corrected p = 0.044; TNF-α: β = −0.41, 95% CI: [−0.72, −0.10], FDR-corrected p = 0.044). These results did not change when accounting for time of day (IL-6: uncorrected p = 0.009; TNF-α: uncorrected p = 0.012). FA in the other tracts (CCS, left UF, and right UF) were not significantly associated with IL-6 or TNF-α (all FDR-corrected *ps* > 0.312). See Fig. 4 and Table 2 for more details. Diagnostic group did not moderate the associations between FA of the CCG and IL-6 and TNF-α (all uncorrected *ps* > 0.338). Within the MDD group only, we found comparable effect sizes in the associations between IL-6 and TNF-α and FA of the CCG that did not meet statistical significance (IL-6: β = −0.26, 95% CI: [−0.60, −0.08], uncorrected p = 0.13; TNF-α: β = −0.38, 95% CI: [−0.76, 0.01], uncorrected p = 0.056). Within the CTL group only, we also found similar effect sizes in the associations between IL-6 and TNF-α and FA of the CCG that met statistical significance without correction (IL-6: β = −0.65, 95% CI: [−1.17, −0.14], uncorrected p = 0.017; TNF-α: β = −0.34, 95% CI: [−0.68, 0.00], uncorrected p = 0.05). Diagnostic group did not moderate the associations between FA of the UF and IL-6 and TNF-α (all uncorrected *ps* > 0.376) nor between the FA of the CCS and IL-6 and TNF-α (all uncorrected *ps* > 0.841). These results did not change when accounting for time of day as an additional covariate.

3.4. Sensitivity and post hoc analyses

To test the robustness of the association between CCG with IL-6 and TNF-α, we reran our models with psychiatric and non-psychiatric medication use, and motion during the scan as additional covariates. These analyses did not change the significance of our finding that higher levels of IL-6 were significantly associated with lower FA in the CCG (β = −0.34, 95% CI: [−0.63, −0.05], p = 0.024); however, the inclusion of these covariates did reduce the significance of the effect of TNF-α (β = −0.34, 95% CI: [−0.68, 0.00], p = 0.05). We also examined whether other diffusivity metrics (AD, MD, RD) of the CCG were also associated with IL-6 and TNF-α. We found that higher levels of these peripheral cytokines were significantly associated with lower AD in the CCG (IL-6: β = −0.34, 95% CI: [−0.61, −0.06], p = 0.016; TNF-α: β = −0.37, 95% CI: [−0.67, −0.07], p = 0.018), but were not associated with MD or RD in the CCG (all *ps* > 0.366).

Finally, we tested whether FA in 41 of the white matter tracts examined in van Velzen et al. (2020) showed any associations with IL-6 and TNF-α. Higher peripheral inflammation was associated with lower FA in the left and right external capsule (all βs < −0.42, all uncorrected *ps* < 0.027), in the right retrolenticular portion of the internal capsule (all βs < −0.42, all uncorrected *ps* < 0.021), and in the left superior fronto-occipital fasciculus (all βs < −0.48, all uncorrected *ps* < 0.008). A full summary of these findings can be found in the Supplement and in Tables S5 and S6.

4. Discussion

Depression often emerges during adolescence and has been linked with both elevated levels of inflammation and alterations in fronto-cingulate-limbic brain circuits. Therefore, the present study was conducted to elucidate the extent to which peripheral concentrations of inflammatory cytokines may relate to alterations in white matter microstructure in the CC and UF—two white matter tracts that have been shown consistently to be associated with depression—in adolescents with and without MDD. As we hypothesized, depressed adolescents had higher peripheral levels of both IL-6 and TNF-α pro-inflammatory cytokines than did psychiatrically healthy adolescents. Moreover, higher levels of these pro-inflammatory cytokines were associated with lower FA (and lower AD) in the CCG across the entire sample. Surprisingly, we did not find significant associations between

Table 1
Descriptive statistics for demographic and primary variables of interest in the full sample and by diagnosis. Significance values in the final column indicate whether there are diagnostic group differences. χ^2 tests were used to examine group differences in categorical variables and Student's *t*-tests (assuming homogeneity of variance) were used to examine group differences in continuous variables. Motion refers to the average amount of movement across six dimensions during the diffusion MRI scan, where negative values refer to displacement in the leftward direction for x, the posterior direction for y, the inferior direction for z, leftward tilt for pitch, counterclockwise rotation for roll, and downward tilt for yaw. All ranges reported indicate minimum and maximum values. BMI = body mass index; FA = fractional anisotropy; MFI = mean fluorescence intensity; PI = Pacific Islander; SD = standard deviation.

	Total ($N = 75$)	CTL ($N = 19$)	MDD ($N = 56$)	*p*-value
Gender				0.493
Male	30 (40.0%)	9 (47.4%)	21 (37.5%)	
Female	42 (56.0%)	10 (52.6%)	32 (57.1%)	
Non-binary	3 (4.0%)	0 (0.0%)	3 (5.4%)	
Sex				0.448
Male	30 (40.0%)	9 (47.4%)	21 (37.5%)	
Female	45 (60.0%)	10 (52.6%)	35 (62.5%)	
Ethnicity				0.108
Hispanic or Latino	13 (17.3%)	1 (5.3%)	12 (21.4%)	
Non-Hispanic or Latino	62 (82.7%)	18 (94.7%)	44 (78.6%)	
Race				0.456
American Indian or Alaska Native	2 (2.7%)	0 (0.0%)	2 (3.6%)	
Asian	16 (21.3%)	7 (36.8%)	9 (16.1%)	
Black or African American	2 (2.7%)	0 (0.0%)	2 (3.6%)	
Native Hawaiian or PI	0 (0.0%)	0 (0.0%)	0 (0.0%)	
White	36 (48.0%)	8 (42.1%)	28 (50.0%)	
Multiracial	13 (17.3%)	3 (15.8%)	10 (17.9%)	
Other	6 (8.0%)	1 (5.3%)	5 (8.9%)	
Age				0.349
Mean (SD)	16.16 (1.23)	15.93 (1.03)	16.23 (1.28)	
Range	13.59–18.39	13.59–17.44	13.95–18.39	
Parental Education Level				0.162
# missing/declined to report	4	0	4	
Less than a high school diploma	0 (0.0%)	0 (0.0%)	0 (0.0%)	
High school graduate or equivalent (GED)	1 (1.4%)	0 (0.0%)	1 (1.9%)	
Some college, no degree	6 (8.5%)	0 (0.0%)	6 (11.5%)	
Associate's degree (e.g. AA, AS)	1 (1.4%)	0 (0.0%)	1 (1.9%)	
Bachelor's degree (e.g. BA, BS)	17 (23.9%)	2 (10.5%)	15 (28.8%)	
Master's degree (e.g. MA, MS)	30 (42.3%)	10 (52.6%)	20 (38.5%)	
Doctoral or professional degree (MD, DDS, DVM, PhD, EdD)	16 (22.5%)	7 (36.8%)	9 (17.3%)	
Psychiatric Medication Status				<0.001
Yes	47 (62.7%)	19 (100.0%)	28 (50.0%)	
No	28 (37.3%)	0 (0.0%)	28 (50.0%)	
Non-Psychiatric Medication Status				0.987
Yes	71 (94.7%)	18 (94.7%)	53 (94.6%)	
No	4 (5.3%)	1 (5.3%)	3 (5.4%)	
Tanner Score				0.097
# missing/declined to report	7	0	7	
Mean (SD)	4.419 (0.57)	4.237 (0.65)	4.490 (0.52)	
Range	3–5	3–5	3–5	
RADS Total Score (raw)				< 0.001
Mean (SD)	71.75 (18.90)	46.42 (10.83)	80.34 (12.06)	
Range	31–112	31–71	57–112	
CDRS *t*-score				< 0.001
Mean (SD)	58.98 (17.00)	32.50 (4.33)	67.97 (7.62)	
Range	30–86	30–42	51–86	
BMI				0.124
# missing/declined to report	1	0	1	
Mean (SD)	22.76 (4.90)	21.27 (2.66)	23.28 (5.38)	
Range	14.75–38.51	18.11–27.81	14.75–38.51	
IL-6 (MFI)				0.004
# missing	15	2	13	
Mean (SD)	4.566 (0.19)	4.455 (0.18)	4.610 (0.18)	
Range	4.134–5.04	4.13–4.75	4.20–5.04	
TNF-α (MFI)				0.011
# missing	15	2	13	
Mean (SD)	4.840 (0.24)	4.715 (0.21)	4.889 (0.24)	
Range	4.21–5.48	4.21–5.04	4.41–5.48	
IL-6 (pg/mL)				0.007
# missing	15	2	13	
Mean (SD)	42.16 (18.40)	32.11 (8.44)	46.14 (19.78)	
Range	18.40–123.49	18.40–48.04	20.82–123.49	
TNF-α (pg/mL)				0.045
# missing	15	2	13	
Mean (SD)	60.92 (73.00)	31.03 (7.71)	72.73 (83.42)	
Range	15.23–334.19	15.23–45.79	20.51–334.19	
Assay Batch				
# missing	15	2	13	0.209
02/1/2019	1 (1.7%)	0 (0.0%)	1 (2.3%)	

(*continued on next page*)

Table 1 (*continued*)

	Total ($N = 75$)	CTL ($N = 19$)	MDD ($N = 56$)	*p*-value
10/19/2020	53 (88.3%)	17 (100.0%)	36 (83.7%)	
06/21/2021	6 (10.0%)	0 (0.0%)	6 (14.0%)	
Motion				0.856
Mean (SD)	−0.05 (0.05)	−0.05 (0.06)	−0.05 (0.05)	
Range	−0.16–−0.09	−0.15–0.05	−0.16–0.09	
Scan after upgrade post-COVID-19				0.137
No	69 (92.0%)	19 (100.0%)	50 (89.3%)	
Yes	6 (8.0%)	0 (0.0%)	6 (10.7%)	
Left Uncinate Fasciculus FA				0.216
# missing	2	0	2	
Mean (SD)	0.44 (0.04)	0.43 (0.05)	0.44 (0.03)	
Range	0.35–0.53	0.35–0.53	0.37–0.52	
Right Uncinate Fasciculus FA				0.294
Mean (SD)	0.43 (0.03)	0.43 (0.03)	0.44 (0.03)	
Range	0.34–0.49	0.38–0.48	0.34–0.49	
Left UF Tract Length (mm)				0.603
# missing	2	0	2	
Mean (SD)	3653.71 (1919.26)	3852.61 (2404.12)	3583.73 (1738.37)	
Range	1261.50–10542.00	1261.50–10343.00	1572.0–10542.00	
Right UF Tract Length (mm)				0.114
Mean (SD)	2222.21 (1395.40)	2660.45 (2183.01)	2073.52 (985.55)	
Range	641.03–1007.80	721.90–1007.80	641.03–5333.50	
Corpus Callosum Genu FA				0.371
Mean (SD)	0.56 (0.02)	0.55 (0.02)	0.56 (0.02)	
Range	0.50–0.60	0.51–0.59	0.51–0.60	
Corpus Callosum Splenium FA				0.009
# missing	1	0	1	
Mean (SD)	0.63 (0.03)	0.62 (0.03)	0.64 (0.03)	
Range	0.55–0.69	0.55–0.66	0.55–0.69	
Corpus Callosum Genu Tract Length (mm)				0.084
Mean (SD)	4286.29 (2542.84)	5158.34 (3173.96)	3990.42 (2247.44)	
Range	1246.70–1327.40	1396.40–1310.90	1246.70–1327.40	
Corpus Callosum Splenium Tract Length (mm)				0.952
Mean (SD)	6849.74 (5045.44)	6910.23 (4980.36)	6829.21 (5111.85)	
Range	2616.00–29077.00	2616.00–21550.00	2672.6–29077.00	

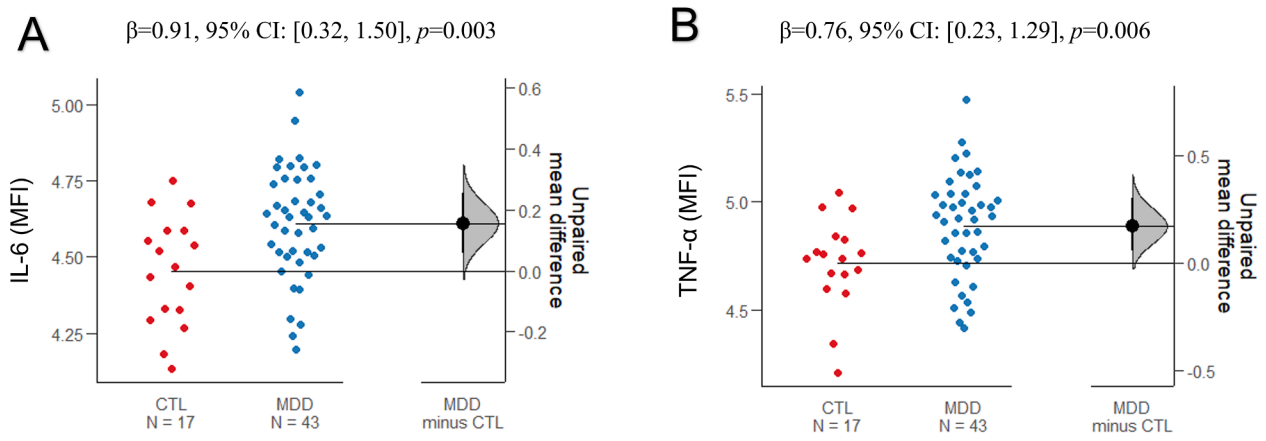

Fig. 3. Group differences in interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). All data displayed without adjustment covariates for visualization only.

pro-inflammatory cytokines and FA of the UF, nor did we find that diagnostic group moderated the significant association between peripheral levels of pro-inflammatory cytokines and white matter microstructure of the CCG. It is likely that the relatively small sample size of our investigation limited our ability to detect these associations. Nevertheless, this study is the first to demonstrate a significant association between depression-related pro-inflammatory cytokines and diffusivity metrics in tracts in human adolescents.

Previous studies in non-clinical samples of adolescents have identified robust associations between peripheral levels of inflammatory markers, including IL-6 and TNF-α, and resting-state functional connectivity in fronto-cingulate-limbic networks (Nusslock et al., 2019; Swartz et al., 2021). Specifically, in an adolescent cohort (ages 13–14 years) exposed to early adversity, Nusslock et al. (2019) reported that higher levels of inflammation (using a composite score comprising of C-reactive protein, IL-6, IL-10, and TNF-α) were associated with lower resting-state functional connectivity in a fronto-temporal network composed mostly of regions in the inferior frontal gyrus and middle temporal gyrus. Similarly, in a community sample of adolescents (ages 12–15 years), Swartz et al. (2021) recently reported that higher levels of TNF-α were associated with stronger resting-state functional connectivity between the right amygdala and the left striatum (ventral striatum, caudate, and putamen), as well as with lower resting-state functional connectivity between the right inferior frontal gyrus and left parietal cortex. Although neither of these investigations recruited adolescents on the basis of depression (in fact, Swartz et al. did not find that levels of inflammation were dimensionally associated with self-reported depression symptoms, which may have been due to the relatively limited

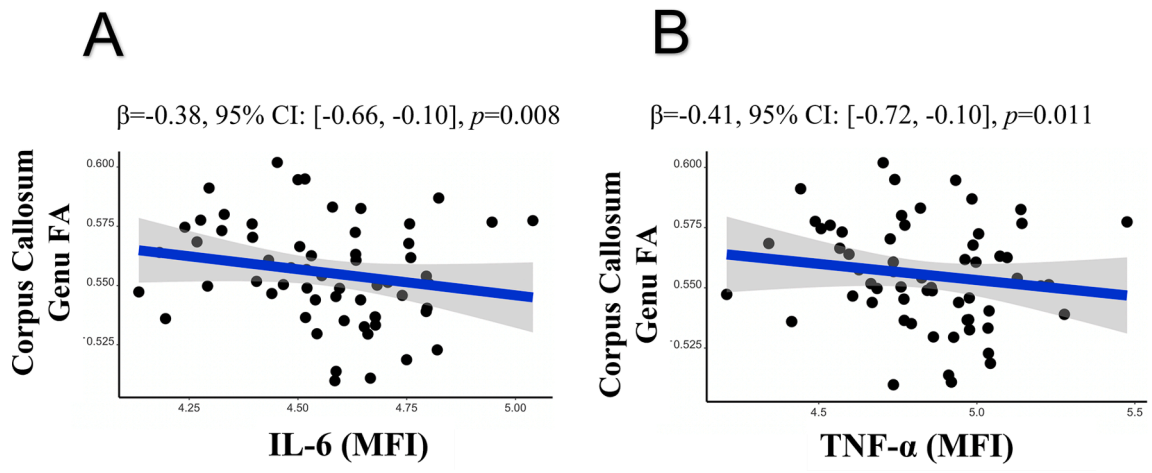

Fig. 4. Linear associations between interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) and fractional anisotropy (FA) of the corpus callosum genu. All data displayed without adjustment covariates for visualization only. See Table 2 for more details.

Table 2
Summary of estimated linear associations between peripheral cytokines and tract fractional anisotropy (FA). In all models, age, batch, BMI, diagnostic group, gender, scan timepoint, and tract length were included as covariates. All reported beta coefficients are standardized. See Table S2 for results using calculated concentration values (pg/mL) instead of mean fluorescence intensity (MFI) values. CI = confidence interval; FA = fractional anisotropy; FDR = false discovery rate; SE = standard error.

CORPUS CALLOSUM GENU FA								
Cytokine	Beta Coefficient	SE	95% CI	*t*(49)	*p*-value	*p*-value (FDR-corrected)	R^2	Adjusted R^2
IL-6	−0.38	0.14	[−0.66, −0.10]	−2.75	0.008	0.044	0.288	0.133377836
TNF-α	−0.41	0.16	[−0.72, −0.10]	−2.64	0.011	0.044	0.281	0.124753741
LEFT UNCINATE FA								
Cytokine	**Beta Coefficient**	**SE**	**95% CI**	***t*(49)**	***p*-value**	***p*-value (FDR-corrected)**	**R^2**	**Adjusted R^2**
IL-6	−0.18	0.15	[−0.47, 0.11]	−1.22	0.227	0.312	0.218	2.97E-02
TNF-α	−0.2	0.16	[−0.53, 0.13]	−1.21	0.234	0.312	0.218	0.029
RIGHT UNCINATE FA								
Cytokine	**Beta Coefficient**	**SE**	**95% CI**	***t*(49)**	***p*-value**	***p*-value (FDR-corrected)**	**R^2**	**Adjusted R^2**
IL-6	−0.18	0.15	[−0.47, 0.11]	−1.22	0.229	0.312	0.232	0.02941961
TNF-α	−0.25	0.16	[−0.58, 0.07]	−1.57	0.123	0.312	0.247	0.04775091
CORPUS CALLOSUM SPLENIUM FA								
Cytokine	**Beta Coefficient**	**SE**	**95% CI**	***t*(48)**	***p*-value**	***p*-value (FDR-corrected)**	**R^2**	**Adjusted R^2**
IL-6	−0.08	0.15	[−0.38, 0.22]	−0.54	0.594	0.678	0.175	5.95E-03
TNF-α	−0.05	0.17	[−0.39, 0.29]	−0.27	0.787	0.787	0.171	1.53E-03

range of depressive symptoms in that cohort), these findings are broadly consistent with the present results in implicating fronto-cingulate-limbic circuits and, specifically, cross-hemispheric connections (as reported by Swartz et al., 2021). Thus, the present study advances our understanding of the potential impact of peripheral inflammation on brain circuits by demonstrating that the CCG—which connects left and right frontal and cingulate cortices and undergirds a wide range of affective and cognitive processing—is an important neurobiological target of inflammatory signals that may, in turn, reflect vulnerability to the onset of depression in adolescence.

Even though we did not find significant associations between pro-inflammatory cytokines and white matter microstructure of the UF or CCS, the effect sizes we observed for TNF-α specifically suggest that with a larger sample size, it is likely that FA in these tracts would also show a significant negative association with TNF-α. Given the scope of the parent project (see Walker et al., 2020, for a more detailed protocol) and the disruptions faced by the COVID-19 pandemic, we were limited in our ability to acquire additional data. Nevertheless, our results provide a strong scientific rationale for larger studies in the future that are adequately powered to confirm and characterize the magnitude of the associations between inflammatory markers and diffusivity metrics in other depression-relevant white matter tracts.

We also did not find diagnostic group differences in FA in any of the tracts of interest. Our sample size may have limited our ability to detect the subtle effect sizes in FA of the CCG and UF, although a recent harmonized meta-analytic study of diffusion MRI studies comparing 372 adolescents with MDD and 290 CTL did not detect any robust group differences in diffusivity (van Velzen et al., 2020). Therefore, our data, as well as recent meta-analytic evidence (Toenders et al., 2021), indicate that levels of peripheral inflammation (notably IL-6 and TNF- α) may be stronger indicators of MDD in adolescents than are FA and related metrics. Nevertheless, our results have significant clinical implications for the treatment of adolescent MDD. Considering the influence of individual cytokines, TNF-α has been posited to have a serotonin-reducing effect by activating the HPA axis (Kaster et al., 2012), leading to downstream activation of indoleamine-2,3-dioxygenase on the surface of macrophages and dendritic cells in the brain (Yang et al., 2015). In turn, indoleamine-2,3-dioxygenase, through the kynurenine pathway, can catabolize tryptophan, a substrate for the synthesis of serotonin (Dantzer et al., 2008). Similarly, IL-6 may also be implicated in the

inhibition of hippocampal neurogenesis, which is hypothesized to underlie antidepressant treatments of depression (Sahay and Hen, 2007; Spalding et al., 2013). Interestingly, IL-6 deficiency in mice confers resistance to the development of depressive symptoms induced by stress (Chourbaji et al., 2006) whereas unsuccessful responses to serotonin-selective reuptake inhibitors and other traditional antidepressants have been associated with higher peripheral levels of inflammation (Liu et al., 2020). Given that the upregulation of indoleamine-2,3-dioxygenase and engagement of the kynurenine pathway generates the metabolic biproduct quinolinic acid, a potent N-methyl-D-aspartate receptor glutamatergic agonist, and that cytokines in the central nervous system also degrade excitatory amino acid transporters, which are almost solely responsible for the reuptake of synaptic glutamate into astrocytes, our findings provide support for theories that anti-inflammatory and/or glutamatergic strategies may be useful interventions or adjunctive options for the treatment of adolescent depression (Köhler et al., 2014; Toenders et al., 2021).

A limitation of the present study is that depressed adolescents who were interested in participating in our study continued their treatment with medications, and may have had psychiatric comorbidities, including anxiety disorders and attention deficit and hyperactivity disorder. While such inclusion criteria allowed us to assess a more generalizable sample of depressed adolescents who are representative of those found in clinics, future investigations may choose to recruit more clinically homogenous samples so as to more precisely examine links between inflammation and depression-specific phenotypes. Although our results did not change when accounting for time of day, we did not acquire detailed information on time since wakening, hours of sleep, circadian misalignment, or other more precise measures of potential circadian effects and sleep quality on inflammatory markers. Future studies should collect sleep-related information to account for these potential confounds. Our study also focused exclusively on specific fronto-cingulate-limbic white matter tracts that have previously been linked with adolescent depression (LeWinn et al. 2014). However, our exploratory whole-brain analysis revealed that striatal white matter connections appear to be sensitive to peripheral inflammation, consistent with previous work in functional MRI examining associations between inflammation and reward-related activation in the striatum (Brydon et al., 2008; Capuron et al., 2012; Eisenberger et al. 2010; Miller et al., 2021). We also found that higher levels of inflammation were associated with lower FA in the left superior fronto-occipital fasciculus, a tract that may be related to attention and decision-making (although the existence of this tract in humans remains controversial; see Bao et al., 2017). Future studies are needed to replicate these effects. Given emerging work indicating that elevated inflammation characterizes specific subtypes of depression (Felger & Miller, 2020; Haroon et al, 2018; Moriarity et al., 2021; Lindqvist et al., 2017), it is also important to examine whether certain subtypes of depression, or clusters of symptom presentations, may have an especially pronounced association between inflammatory profiles and brain connectivity. It will also be important for future longitudinal studies to examine the effect of developmental changes in inflammatory profiles in depressed (and nondepressed) adolescents and determine the degree to which these changes affect developmental changes in white matter microstructure in fronto-cingulate-limbic tracts. Finally, experimental studies that manipulate peripheral levels of inflammation over a period sufficient to invoke changes in white matter in fronto-limbic-tracts (e.g., stress reduction interventions that lower levels of inflammation, see Dutcher et al., 2021) may elucidate the acute versus enduring effects of inflammation on brain connectivity during this critical developmental period.

In sum, the present study is the first to examine associations between circulating levels of IL-6 and TNF-α and structural brain connectivity, assessed with white matter microstructure, in depressed and psychiatrically healthy adolescents. Our results suggest that peripheral levels of inflammation are associated with fronto-cingulate-limbic tract connectivity that underlies critical cross-hemispheric connections, supporting a wide range of affective and cognitive processing implicated in depression. Although our findings require independent replication (particularly for our results with TNF-α, which may not show as strong of an association with plasma levels based on our protocols and/or assay kit), this study nevertheless provides evidence for the contribution of inflammation to neurophenotypes associated with adolescent depression and may inform the development of novel clinical interventions that target neuroimmune pathways in this disorder.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2021.12.003.

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