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Divyaansh Raj March 26, 2020

Does Inflammation Damage White Matter Tracts in Patients with Major Depression

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Goal: This study investigated whether white matter integrity is related to measures of disease chronicity and severity. Further, inflammatory cytokines interleukin six's and C-reactive Protein's correlation with white matter integrity was examined. This study also explored whether gender modulates the inflammation effect.
Methods: Diffusion Tensor Imaging (DTI) and Tract Based Spatial Statistics were conducted on 125 treatment-naïve patients to create generalized linear models (GLM) exploring the relationship between severity, chronicity, white matter integrity as measured by four common DTI indices, and inflammation.
Results: Results indicated significant negative correlations between white matter integrity and disease chronicity and severity, particularly in the corpus collosum, subgenual cingulate cortex (Brodmann Area 25), uncinate fasciculus, superior longitudinal fasciculus, and forceps minor. There were no significant correlations between IL6 or CRP and the DTI measures in the total sample set or male-female subgroups. Splitting the population into chronic and non-chronic patients revealed that in Brodmann Area 25 white matter integrity was decreased in chronic patients and increased in non-chronic patients.
Conclusions: These results suggest that white matter pathology contributes to the pathophysiology of depression and is related to the severity and chronicity of illness. Although there were no significant relationships between IL6 or CRP and DTI indices in the total sample, the results suggest that inflammation-induced white matter pathology may contribute to the disease process in MDD patients with a chronic course of illness.

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Abstract

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Conclusions: These results suggest that white matter pathology contributes to the pathophysiology of depression and is related to the severity and chronicity of illness. Although there were no significant relationships between IL6 or CRP and DTI indices in the total sample, the results suggest that inflammation-induced white matter pathology may contribute to the disease process in MDD patients with a chronic course of illness.

Background:

Major Depressive Disorder (MDD) has a lifetime prevalence of 20% in individuals and is the third leading cause of disability world-wide (World Health Organization, 2017). Further, with more than 30% of patients failing to respond to multiple kinds of treatments, it is critical to better understand the pathophysiology of MDD in order to tailor more effective treatments and identify meaningful biomarkers to identify likely responsiveness of patients. The neurobiology of MDD still remains poorly understood, however. Previous literature increasingly implicates the neurocircuitry of important mood regulating regions in the progression of MDD (Choi et al., 2014). In this model, the network of mood regulating regions is dysfunctional. Rajkowska and colleagues' influential review expands on this possible network dysfunction through identifying potential biological pathologies that could contribute to network dysfunction. Their meta-analysis identifies a possible pathophysiology of oligodendroglia underlying depression. They most importantly underscore a gap in knowledge in how gliogenic and gliotoxic factors impact oligodendroglial pathophysiology at the onset of depression (Rajkowska et al., 2010).

Mounting evidence suggests that chronic inflammation has an etiological role in the pathogenesis of many neurological and neuropsychiatric diseases, including a subtype of MDD (Raison et al., 2005). Environmental stressors, such as physical and psychological stress, can activate the immune system in the central nervous system and peripherally to induce the release of inflammatory cytokines from white blood cells and C-reactive protein (CRP) from the liver (Felger et al. 2013). Stress-induced inflammatory cytokines produce both behavioral and biological effects. Chronic peripheral infusion of inflammatory cytokines, used to treat conditions such as hepatitis C, induces anhedonia, decreased social action, and a plethora of neuropsychiatric symptoms (Capuron and Miller 2004). CRP and certain inflammatory

cytokines, most prominently interleukin-6 (IL6), have also been shown to be positively associated with depression severity (Raison et al., 2005), and may differ by sex, with women being more susceptible to the effects of cytokines on mood symptoms (Jha 2019). Lastly, patients with chronic medical illnesses associated with elevated pro-inflammatory cytokines, such as cancer and HIV, develop comorbidities of depression, fatigue, and psychomotor slowing at rates approaching 50% (Musselman et al., 2001, Felger et al. 2013).

The markers of inflammation most frequently found to be elevated in the serum or plasma of patients with MDD compared to healthy control subjects are IL6 and CRP (Raison et al., 2005). In addition, increases in tumor necrosis factor (TNF-alpha) and (Interleukin 1 Beta) IL-1B have also been found to be elevated in some studies (Raison et al., 2005). Of the central nervous system cell types, oligodendroglia are the most sensitive to an inflammatory cascade. Oligodendroglia provide the myelination of white matter tracts in the brain (Rajkowska et al., 2010). Literature studying multiple sclerosis (MS), a chronic inflammatory disease, has documented the cytotoxic effects inflammatory cytokines can have on the structural integrity of white matter tracts (Buntinx et al., 2014). Axonal demyelination can delay or completely block signal conduction through the affected region and impact the connectivity between two brain regions.

TNF-alpha and IL-1B can induce oligodendroglia cell death in many ways (Buntinx et al., 2004; McTigue et al., 2008; Li et al., 2008). Firstly, TNF-alpha and IL-1B can have a direct toxic effect on oligodendroglia by binding to the p55 TNF receptor of oligodendroglia. Secondly, the inflammatory cascade can lead to glutamate excitotoxicity by down-regulating glutamate uptake and up-regulating glutamate release in astrocytes. Glutamate excitotoxicity decreases neurotrophic support and plasticity and increases oxidative stress in oligodendroglia in human

cell cultures. Oxidative stress culminates in apoptosis and subsequent demyelination of white matter tracts in the brain (Felger et al., 2013, Miller et al., 2009, Buntinx et al., 2004, and McTigue et al., 2008). Consequently, microstructural damage to white matter tracts due to increases in inflammatory cytokines could indirectly lead to impaired neuronal signaling (McTigue et al., 2008).

Although TNF-alpha and IL-1B could be directly responsible for oligodendroglia cell death in vivo, their concentrations are highly transient in the periphery, making them difficult to accurately quantify (Slaats et al., 2016). CRP and IL-6 concentrations are highly correlated with those of TNF-alpha and IL-1B and have a longer peripheral half-life, making them more suitable for measurement as a biomarker for risk of white matter degradation in the brain (Slaats et al. 2016).

An important challenge to determining the biological processes underlying psychiatric disorders is that biopsying the brain in living patients is not ethical. Consequently, methods for imaging the living brain and its activity have been an important development for neuroscientific approaches to illnesses like MDD. Many studies have focused on brain region dysfunction from a functional connectivity lens (Seminowicz et al, 2004; Choi et al, 2014). Functional imaging detects and measures changes in metabolic, electrical or blood flow changes in the brain. Functional connectivity (FC) measures associations in activity across distributed brain regions through functional Magnetic Resonance Imaging (fMRI), either while a subject is at rest or while performing a task (Moonen 1999). The primary form of fMRI uses blood-oxygen level dependent (BOLD) contrast. MRI is able to distinguish between the paramagnetic properties of oxygenated and deoxygenated hemoglobin. A T2 pulsing sequence is used to capture this property through analyzing the change in oxygenated and deoxygenated blood in different areas

in the brain. By this method, fMRI attempts to indirectly measure neuronal activity. FC analysis is able to make the corollary that higher oxygenation is related to higher synaptic activity through the assumption that active neurons have higher metabolic demands for oxygen than non-active neurons. FC, thus, refers to the temporal relationship between changes in blood flow and oxygenation between two areas of the brain and the level of FC is directly related to the Pearson correlation between oxygenation changes in two areas of the brain. Although FC analysis can discern an altered brain connectivity pattern between two or more regions in patients compared to healthy controls, this technique cannot explain what is *causing* the aberrant biological abnormalities in given brain disorders. (Reid et al. 2017). An influential study that examined the impact of inflammation on FC using fMRI found that elevated peripheral IL6 and CRP concentrations were associated with reduced FC between the VMPFC and ventral striatum, but this finding awaits replication (Felger et al., 2016).

An alternative approach to moment-to-moment changes in blood flow measured by fMRI is to explore neurocircuitry via the structural connectivity of the white matter tracts that connect key mood regions (Cole et al., 2012; Zhu et al., 2011; Wu et al., 2012; Korgaonkar et al., 2011). White matter integrity can be assessed using, Diffusion-Weighted Imaging (DWI), an MRI-based imaging tool that measures the degree of movement, or anisotropy, of water in the brain (Alexander 2007). Water moves more freely in gray matter than in white matter because water movement in white mater perpendicular to the fiber direction is hindered by myelin layers and cell membranes compared to diffusion parallel to the white matter (WM) tracts; in grey matter these constraints are not present. DWI uses directional pulsed magnetic field gradients to get a sense of the motion of the protons in water molecules. The most common number of gradients used in a DWI scan is 64. Through these 64 gradients, when T1 and T2 radio waves hit water

molecules in brain, there is a difference in the intensity of signal emitted. The difference in signal can be used to quantify the velocity and location of the movement of protons. The Diffusion Tensor Imaging (DTI) model is commonly used to measure the velocity and location of water molecules and create tensors that are composed of eigenvectors (ε1, ε2, ε3) and eigenvalues (λ1, λ2, λ3) that help to characterize the movement direction of water in a particular area of the brain (Figure 1). Each eigenvector (ε) and eigenvalue (λ) represents a direction and magnitude, respectively, of the water molecule movement in a particular voxel. The resultant tensor formed from the eigenvectors and eigenvalues helps quantify the anisotropy or isotropy of water movement (Figure 2).

Validated measures of WM integrity derived from DTI scans include fractional anisotropy (FA, Equation1), mean diffusivity (MD, Equation 2), radial diffusivity (RD, Equation 3), and axial diffusivity (AD, Equation 4).

FA quantifies the ability of water to move in a specific voxel. A value of zero means that diffusion is isotropic (unrestricted) and a value of one means that the water's movement is anisotropic—or confined to a single direction. Higher FA values therefore indicate greater WM integrity. FA is calculated using Equation 1:

Equation 1:

$$FA = \sqrt{\frac{(\lambda_1-\lambda_2)^2+(\lambda_2-\lambda_3)^2+(\lambda_1-\lambda_3)^2}{2(\lambda_1{}^2+\lambda_2{}^2+\lambda_3{}^2)}}$$

MD is an inverse measure of the membrane density, indicating necrosis or loss of tissue integrity and is calculated by averaging the orthogonal eigenvalues λ1, λ2, λ3.

Equation 2:

$$MD = \frac{\lambda 1 + \lambda 2 + \lambda 3}{3}$$

AD and RD are tools to describe the structural abnormalities, axonal injury, and de/dys-myelination in the region being examined. For these measures, AD represents λ1, the eigenvalue parallel to the anisotropic flow, and RD is the average of eigenvalues λ2 and λ3, which are perpendicular to anisotropic flow. This means AD best represents how well water molecules are moving along (i.e., parallel to) white matter tracts, while RD best measures if water is moving perpendicular to the white matter tract.

Equation 3:

$$RD = \frac{\lambda 2 + \lambda 3}{2}$$

Equation 4:

$$AD = \lambda 1$$

These characteristics and indexes of water movement within white matter tracts allow DTI to characterize the microstructural composition and changes of WM in the brain. FA is one of the most stable measures of microstructural biological changes. Although sensitive to biological changes, FA is not able to characterize the kind of microstructural change that has happened. MD provides an excellent way to index membrane density and is particularly sensitive to cellularity, edema, and necrosis. AD is an excellent way of measuring axonal injury and oligodendroglial pathology. RD is a good indicator of demyelination and changes in axonal diameters (Soares et al. 2013). Since many developmental, aging, and pathological conditions affect the microstructural and architectural integrity of WM tracts, DTI gives researchers the ability to track these factors.

A structural connectivity approach has indicated that white matter abnormalities may be present in MDD subjects and that there is a negative association between white matter integrity and disease severity (Cole et al. 2012). The evidence of WM abnormalities in patients with

depression is variable, however. While some studies (Cole et al., 2012; Zhu et al., 2011; Wu et al., 2012; Korgaonkar et al., 2011) indicate that there are FA abnormalities when comparing healthy controls and patients with MDD, others (Choi et al. 2015) found no WM differences in treatment naïve patients with MDD. Beyond correlations with clinical measures, identifying significant associations between WM integrity and IL-6 and CRP concentrations would support the case that inflammation deleteriously impacts the neural circuitry that connects brain regions affected in MDD. Only one prior study has examined MDD patients for associations between white matter integrity and inflammatory cytokines. The study found a statistically significant inverse correlation between IL-1β levels and FA values in the inferior fronto-occipital fasciculus genu of the corpus callosum, which had significantly lower FA values in patients with MDD than those of healthy controls (Sugimoto et al., 2018). However, with only 35 *drug-naïve* MDD patients and healthy controls, this study is vulnerable to type II errors and requires replication. With most structural studies having 30-50 subject scans in their analysis larger datasets are needed to obtain more reliable estimates of WM pathology in MDD.

Another important clinical factor, previously unexamined for its association with WM integrity, is the chronicity of the depressive episode. Chronicity is associated with poorer response to treatment and greater susceptibility to relapse (Kemp et al. 2008). It is critical to identify possible factors that can not only explain abnormalities in white matter, but also are intrinsically linked to clinical measurements like severity and chronicity.

This study aims to use pre-treatment MRI scans of treatment naïve MDD patients to extract indices of white matter integrity (FA, MD, RD and AD) in order to examine associations with clinical variables and biological markers of inflammation. Due to the increased effect

inflammatory cytokines have on the severity of depression in women, this study aims to explore whether there may be increased changes to their white matter as well.

Hypotheses:

H1. In treatment-naïve patients with MDD, depression severity and episode chronicity will be negatively associated with the integrity of WM tracts as assessed by FA, MD, RA or AD.

H2. The inflammatory cytokines IL6 and CRP will have statistically significant negative correlations with the integrity of WM tracts in important mood-regulating brain networks as assessed by FA, MD, RA or AD.

H3. Gender will moderate the association between inflammatory cytokines and WM integrity.

Methods:

Participants

The Predictors of Remission in Depression to Individual and Combined Treatments (PReDICT) study enrolled adults with treatment-naive MDD between the ages of 18-65. The purpose of the PReDICT study was to identify biological and clinical predictors and moderators of response to cognitive behavior therapy (CBT), the most widely practiced evidenced-based psychotherapy for MDD, or antidepressant medication. Since one of the biggest confounders of response prediction is treatment history, this study enrolled patients with no prior treatment for depression. This population, therefore, has tremendous potential in isolating unadulterated progression of depression. All subjects provided written informed consent for participation and the study was approved by the Emory Institutional Review Board.

At the study screening visit, participants were determined to have a primary psychiatric disorder of MDD without psychotic or catatonic features via administration of the Structured Clinical Interview for DSM-IV (SCID-IV) (First et al., 1995) and a study psychiatrist's interview. The SCID-IV was also used to assess the length of the current major depressive episode and to determine whether a comorbid anxiety disorder was present. An episode of major depression is defined as "chronic" if it has persisted for two years or longer. Severity of the depressive episode was assessed with the Hamilton Depression Rating Scale 17-item version (HDRS-17) (Hamilton, 1960). The HDRS-17 is a clinician-rated interview that assesses a variety of symptoms that may occur during depressive episodes. Scores range from 0-52, with a score $\geqslant$18 considered to represent at least moderate severity. In PReDICT, patients had to score $\geqslant$18 at the screening visit and $\geq$15 at the baseline visit to be eligible for participation (Dunlop et al.,

2012). Other eligibility criteria included the ability to read and converse in either English or Spanish, and women were required to use a medically acceptable form of birth control.

Exclusion criteria included a primary psychiatric disorder other than MDD, or any lifetime history of bipolar disorder, a psychotic disorder, or neurocognitive disorder. Current (past year) obsessive-compulsive disorder, substance dependence, or anorexia nervosa were also exclusionary, as was meeting criteria for substance abuse in the past three months. Medical conditions that could be the cause of depressive symptoms, or which could interfere with interpretation of study results, were also exclusionary, assessed via medical history, physical exam, electrocardiography, laboratory screening, drug tests, and vital signs at the screening visit. Patients with contraindication to MRI, such as metal in the body or severe claustrophobia, were also excluded.

Data Acquisition:

Inflammatory Markers

Patients who met all PReDICT study eligibility criteria underwent phlebotomy for measurement of inflammatory markers at the baseline visit, without regard for time of day or dietary fasting status. Blood samples were collected from antecubital veins into EDTA tubes between 8 am and 4 pm. Within 10 min of being obtained, the EDTA tubes were centrifuged at 4ºC, the plasma was aliquoted into 1 mL samples and frozen at -80ºC. Batched analysis of plasma CRP was analyzed with a Beckman AU480 chemistry analyzer (Beckman Coulter, Brea, CA, USA) and Ultra WR CRP Kit (Sekisui Diagnostics, San Diego, CA, USA) as described (Felger, 2018). Concentrations of IL-6 were assessed in duplicate using multiplex bead-based

assays (R&D Systems, Minneapolis, MN, USA) and analyzed on a MAGPIX CCD imager (Luminex, Austin, TX, USA). Inter and intra-assay coefficients were reliably <10%.

Neuroimaging

A single-shot spin-echo echo-planar diffusion-weighted imaging sequence was used with generalized auto-calibrating parallel acquisition (*R*=2; Griswold et al, 2002). The parameters used for DWI were: FOV=256 × 256; *b* value=1000 s/mm^2; voxel resolution=2 × 2 × 2 mm; number of slices=64; matrix=128 × 128; TR/TE=11 300/104 ms. Sixty non-collinear directions with four non-diffusion-weighted images (*b*=0) were acquired twice: once with phase encoding in the anterior to posterior (A-P) direction and once in the posterior to anterior (P-A) direction. To compensate for susceptibility distortion, both phase up and down images were acquired. High-resolution structural T_1-weighted images were collected using a 3D magnetization-prepared rapid gradient-echo sequence with following parameters: TR/TI/TE=2600/1100/3 ms; voxel resolution=1 × 1 × 1 mm; number of slices=176; matrix=224 × 256.

Quality Control:

Figure 3 displays the pre-processing pipeline of the neuroimaging data. In order to ensure the quality of the raw DWI data, stringent quality control methods were used (Liu et al. 2010). Eddy-current and head-motion artifact correction were done by registering voxels to the baseline b=0 image. To confirm the accuracy of the eddy current and motion correction, manual inspection was performed, and diffusion tensor model was then calculated using the FMRIB Software Library (FSL). Diffusion tensor, eigenvector, and eigenvalue calculations by the tensor fitting model provided by the FMRI Software Library (FSL), and manual inspection of 64

successive slices of each image was done in important WM bundles. Any evidence of abnormal directionality, value, or coloration of DTI images led to exclusion of the subject.

Lastly, images were manually inspected to ensure that skull stripping was performed accurately in the pre-processing pipeline. The skull stripping script helps the computer isolate the brain region from skull in the original DTI image. Ensuring the skull isn't captured in the extracted image and that the script doesn't also remove portions of the brain allows FSL tract-based-spatial-statistics scripts to better normalize brain images of different patients to the same standardized space (Liu et al. 2010).

Tract Based Spatial Statistics (TBSS):

All patient images were first normalized to the MNI152 sample space (Montreal Neurosciences Institute Brain Atlas, 2001) to allow for appropriate voxel-to-voxel comparisons between subjects. FA, RD, MD, and AD maps for each subject were smoothed using a 5-mm FWHM Gaussian isotropic kernel to remove possible error caused by anatomical mismatching of like-brain regions. TBSS was performed by using the aligned FA maps of all subjects to create a mean FA map. This mean FA map was then skeletonized to represent the centers of the tracts that were common among all the subjects. Next, voxels' FA values below the threshold of 0.2 were interpreted as noise and eliminated from the analysis. The aligned and threshold FA skeleton maps were then projected to the mean FA map to guide the analysis to appropriate voxels. The FA skeleton was used to generate mean AD, MD, and RD maps that were analyzed through the randomization methods.

Statistics:

CRP and IL6 levels were correlated with whole brain diffusion measurements using nonparametric permutational statistical tests (Randomise, FSL, FMRIB, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Randomise) to evaluate the relationship of inflammation with WM integrity. Furthermore, diffusion measurements of specific WM bundles within predefined depression networks were correlated with CRP and IL6 to identify inflammation specific WM abnormalities. Nonparametric permutational statistical methods (also known as randomization methods) were used for inference because the null distribution was not known due to the noise in the data. A permutation test has exact control over the desired false positive rate with very simple assumptions. The null distribution was unknown because either the noise in the data did not follow a simple distribution, or because non-parametric statistics were used to summarize the data. In addition to the permutational test, threshold-free cluster enhancement (TFCE, FSL) approach was applied to choose cluster-forming thresholds and family-wise error correction was used for multiple comparison correction.

The following analyses were completed:

- A median split of age was used to validate the TBSS method.
- Depression Severity (HDRS-17 score) was correlated to DTI indexes, with age and gender as covariates.
- The relationships between CRP and IL6 and DTI indexes was assessed across all patients, using age and gender as covariates. TBSS was used to create a generalized linear model that correlated CRP and IL6 values to DTI indexes.

- Patients were split into male and female groups. Then TBSS was used to test the relationship between CRP and IL6 and DTI indexes in each subgroup. Age was used as a covariate.
- A two-sample non-parametric t-test was performed comparing chronic and non-chronic patient subgroups.

Results

Of the 344 patients randomized in PReDICT, 125 had both complete IL6 or CRP values and DTI scans that survived the stringent quality control requirements. The demographic and clinical characteristics of the sample are listed in Table 1.

Using a statistical threshold of $p < .05$, depression severity, as measured by the HDRS-17, differed significantly between men and women with a p-value $p = .0128$.. CRP and IL6 concentrations did not significantly differ between men and women. Chronicity and family history of MDD or bipolar disorder also did not differ significantly between the genders. It is notable that 35.2% of the patients had a chronic episode, nearly double the 20% rate found in community samples of MDD patients (DSM-5, 2013).

Inflammatory Markers

The relationship between CRP and IL6 was also analyzed to see if testing for one inflammatory cytokine could predict the levels of the other. CRP and IL6 showed a statistically significant, positive relationship (Pearson's $r=.38$, $p<.001$), consistent with previous literature (Figure 4).

Figures 5 and 6 show that age was not significantly correlated with IL6 and CRP concentrations, ($r=.05$, $p=.535$; $r=.08$, $p=.327$, respectively).

Another important question to answer was whether CRP and IL6 concentrations were correlated with depression severity. The scatter plots in Figures 7 and 8 show no significant the correlation between CRP andIL6 and HDRS-17 scores ($r= -0.03$, $p=.697$; $r = -0.09$, $p = .3119$, respectively).

Diffusion Tensor Imaging Results

Validation:

The effect of age on WM integrity has been explored and quantified in previous literature (Gunning-Dixon et al. 2010). As age increases, white matter density and myelination decrease. To verify that the FSL methods would produce similar results in the PReDICT sample, a median split was performed to divide the patient population into two groups, one above the age of 38 years and the other below. These two groups were then contrasted using a two-sample t-test. Results, shown in Figures 9 and 10, validated previous age-related effects on white matter in the FA and RD indexes. This means as age increased, FA decreased, and RD increased. RD describes increased water movement perpendicular to the white matter tract and decreased anisotropic water movement along the white matter tract, indicating microstructural dis-integrity. The changes in RD indicate age-related demyelination, which has been extensively described in the literature (Gunning-Dixon et al. 2010).

DTI Associations with Clinical Variables

To evaluate Hypothesis 1, depression severity, as measured by the HDRS-17, was explored for its relationship to white matter integrity indices, FA, MD, RD and AD, adjusting for age and sex. Figure 11 indicates that as HDRS-17 scores increased, FA values for voxels in these clusters went down—indicating a decrease in microstructural integrity in the following regions: left subgenual cingulate cortex (Brodmann's area 25 (BA 25)), left uncinate fasciculus, right superior longitudinal fasciculus, right and left inferior longitudinal fasciculus, and the right and left inferior fronto-occipital fasciculus. These clusters were significantly correlated with a $p < .05$.

The RD results were also significantly ($p < .05$) correlated with depression severity, though in different tracts than those identified using FA (Figure 12). The correlation of depression severity with RD measures had significant voxel clusters along the corpus callosum, forceps minor and major.

There were no significant correlations of HDRS-17 scores with AD and MD measures.

To evaluate the impact of major depressive episode duration, the sample was separated into two groups: patients with chronic (n=44) versus non-chronic (n=81) current episodes. The FSL program Randomise was used to run a non-parameterized two-sample t-test comparing these two groups. Results indicated unilateral, significant ($p < .05$) differences in RD measures between chronic and non-chronic patients. The right subgenual cingulate cortex (BA 25), forceps minor, right corticospinal tract and right anterior thalamic radiation were identified as areas where radial diffusivity was higher in chronic patients than non-chronic patients (Figure 13). There were no significant differences in AD, MD, or FA between the chronic and non-chronic subgroups.

DTI Associations with Inflammatory Markers

To assess Hypothesis 2, the correlation of IL6 and CRP levels with DTI indexes was explored using a generalized linear model. Contrary to the hypothesis, the TBSS adjusted for age and gender revealed no significant areas of correlation with Il6 or CRP values.

Hypothesis 3 was also not supported, as the analysis in subgroups of men and women also revealed no significant areas of correlation with IL6 or CRP values.

The negative associations of inflammation in the overall sample led to consideration of whether the chronically depressed sample may be more likely to demonstrate WM abnormalities. The inflammation hypothesis of MDD assumes that chronic low-grade inflammation is responsible for maintaining the depressive state (Miller et al. 2009). Thus, it is possible that chronically ill patients' white matter may be uniquely affected by inflammatory cytokines.

Consequently, inflammation levels in chronic and non-chronic patients were compared with a Mann Whitney U test. The IL6 concentration different significantly between chronic and non-chronic patients (1.77 ± 1.53 vs 1.37 ± 1.4, respectively, $p=.026$). CRP levels did not significantly differ between the groups (2.06 ± 1.96 vs 3.68 ± 3.69, respectively, $p=.96$)

A GLM was used to evaluate the relationship of the DTI indexes with CRP and IL6 concentrations within the chronic MDD group.

Figure 14 indicates a single region of correlation between FA and CRP levels. The left subgenual cingulate cortex in BA 25 demonstrated an inverse correlation ($p<.05$) between CRP concentration FA levels. There were no significant correlations with IL6 or CRP with MD, RD, and AD values.

The same GLM analysis was then performed to examine the effects of inflammation within the sample of non-chronic MDD patients. This analysis revealed a negative association between AD and IL6 bilaterally in the cerebro-spinal tract (Figure 15). Surprisingly, the non-chronic patients had a positive correlation between CRP and FA values bilaterally in Brodmann's area 25 ($p < .05$).

The results of all these analyses are summarized in Table 2.

Discussion:

This analysis aimed to evaluate whether markers of inflammation (CRP and IL6) were associated with indices of WM integrity in 125 patients with treatment naïve MDD. Despite previous reports of inflammation causing damage to oligodendroglia in translational (Kumar et al. 2014) and in-vitro studies (Rajkowska et al. 2007), no association was found between CRP or IL6 and the FA, MD, RD and AD indices of WM integrity values, either in the entire sample or the subgroups of males and females. However, in the subset of patients with major depressive episodes that had persisted for 2 years or longer (i.e., those with chronic episodes), a statistically significant inverse correlation was found between CRP and FA in the left subcallosal cingulate cortex (BA25). This association indicates that chronically depressed patients have significantly more microstructural damage to WM (Soares et al. 2013) in a core brain region implicated in the pathophysiology of MDD than patients with episodes lasting less than two years.

The finding that inflammation was associated with reduced WM integrity in chronically depressed patients has face validity, as the inflammation hypothesis rests on the assumption of chronic low-grade inflammation passing through the blood brain barrier over time to activate microglia causes a neurotoxic cascade (Felger et al., 2018). It is important to note, however, that this study had only a single baseline measure of inflammation and, therefore, cannot prove that the inflammation was persistent in the chronic episode patients. Longitudinal studies will be necessary to confirm the effect of chronic inflammation on WM tracts in chronic MDD patients.

Another important result from this analysis was that depression severity was significantly correlated with FA and RD indexes of WM integrity. The regions of WM identified from the FA analysis included the left BA25, left uncinate fasciculus, right superior longitudinal fasciculus (SLF), right and left inferior longitudinal fasciculus (ILF), and the right and left inferior fronto-

occipital fasciculus (IFOF), all of which demonstrated an inverse correlation between FA value and HDRS-17 score.

It is particularly noteworthy that the reduced FA in BA25 was detected both in the correlational analyses of depression severity in the entire sample and in the association with inflammation among the chronically ill patients. The subcallosal cingulate cortex (SCC) in BA25 is the target for deep brain stimulation treatment resistant depression. BA25 serves as an important node in the circuitry of mood regulation and its functional hyperactivity has been reliably identified in highly treatment resistant MDD patients (Mayberg 2009). Tractography studies done by Riva-Posse and colleagues similarly found that the optimal stimulation was impacted by 3 major white bundle tracts that run through the SCC area: uncinate fasciculus, forceps minor, and cingulum bundle—white matter tracts that have been additionally implicated in this study (Riva-Posse et al. 2015). Electrical modulation of the "hub" between these three WM tracts can induce clinical response. The significant results found in this location suggest that the inflammation hypothesis may uniquely contribute to pathology in this region and supporting the SCC as a target for deep brain stimulation.

The other regions identified in the depression severity analysis are also of interest. Similar to Cole and colleagues' 2012 study on disease severity and WM, disease severity was negatively correlated with white matter integrity. The uncinate fasciculus, superior longitudinal fasciculus, and corpus collosum were all regions, in particular, that were found to have decreased WM integrity both in the earlier and the current study.

The uncinate fasciculus (UF) connects the inferior frontal gyrus and the anterior portions of the temporal lobe, including key components of the limbic system, such as the amygdala, hippocampus, and parahippocampus. The UF has been implicated in depression circuitry when

comparing healthy controls and depressed patients (Chen-Han et al. 2016) but hasn't been related previously to depression severity. The location of this finding in the left UF matches the location and corroborates the results published by Chen-Han and colleagues (2016).

The SLF is a bidirectional white matter tract that connects all four lobes of the brain. Importantly, a negative correlation of SLF WM integrity with depression severity emerged in a previous meta-analysis of white matter abnormalities associated with MDD (Murphy et al. 2011), so the current study result is an important confirmation of prior work. Chen-Han and colleagues found that lesions in the SLF were correlated with decreased cognitive control of emotional response as well (Chen-Han et al. 2016). Also notable about the SLF is its connectivity to the dorsolateral and ventral-lateral prefrontal cortex, particular BA 9 and BA 46 (Makris et al. 2005)—areas important for cognition and emotion regulation, which are frequently impaired in patients with MDD and have been implicated in FC analyses of MDD (Mayberg et al 2009).

The ILF connects the visual cortex to the amygdala and has an important role in processing emotion (Herbert et al. 2018). TBI based lesions in the ILF have resulted in hypo-emotionality and flat affect in patients (Bauer et al 1982). The IFOF connects the occipital and temporal lobe and lies just superior to the ILF (Herbert et al. 2018). The IFOF is critical for detecting and processing facial emotions. Lesions in this tract have been shown to decrease sensitivity and recognition for faces exhibiting negative emotions (Crespi et al. 2014).

The RD analyses of depression severity also identified significant correlations with several regions relevant to MDD, though the regions did not overlap with the results from the FA analyses. RD was positively correlated with HDRS-17 scores in the corpus collosum, forceps minor and forceps major. RD, as previously mentioned, is a compelling index for de- or dys-

myelination in white matter. The corpus callosum has been consistently cited as critical to mood regulation and affective processing (Riva-Posse et al. 2015). The implication of the forceps minor is of particular interest because of the critical role this WM tract plays in the depression circuit. Previous literature by Gobbi and colleagues found that MS lesions in the forceps minor region were statistically correlated with an increased occurrence of depression (Gobbi et al. 2014). Furthermore, tractography analysis of this region in depressed patients treated with DBS revealed the forceps minor as a key bundle that predicts response to DBS treatment (Riva-Posse et al. 2015).

The other clinical variable to emerge from the DTI analyses was chronicity of illness. The primary aim of this study was to evaluate the effects of inflammation on WM integrity in BA25, which was detected only in the chronically depressed patients, was previously discussed. However, chronicity alone was also associated with reduced WM integrity in the subgenual cingulate cortex (BA 25), though on the right side, rather than the left that emerged in the inflammation analysis. Other WM tracts identified in the chronic versus non-chronic analysis included forceps minor (also found in the severity analysis), right corticospinal tract (CST) and right anterior thalamic radiation (ATR). As previously mentioned, BA25 and the forceps minor have been consistently implicated in mood regulation and depression circuity (Mayberg 2009; Riva-Posse 2015). Oligodendroglia in the right CST myelinate cortical and motor neurons that interface with interneurons and effector muscles that control motor function. Abnormalities in CST have been previously reported and may explain the psychomotor slowing and agitation that are frequently present in patients with MDD (Sacchet et al. 2014). The right ATR connects the anterior thalamus to the frontal lobe. Chen-Han and colleagues have also found that lower white matter integrity in the ATR accompanies MDD (Chen-Han et al. 2016). Thus, the chronicity

analyses replicate previous findings in the literature, though prior studies did not indicate the results were present only in chronically ill patients. The current results suggest that these decreases in WM integrity may occur primarily in patients with more persistent forms of depression. Another important aspect of the results is that the significant regions in the chronically ill patients were present only on the right side of the brain. This finding might be explained by the right hemisphere's functional dominance in emotional processing and cognition (Gainotti 2019). Linking decreased structural connectivity in important mood regulating regions to the chronicity of a patient's illness has face validity and warrants further research.

The results from the non-chronic patients were unexpected, but also interesting. Firstly, a significant negative correlation was identified between AD and IL6 in the CST. The most interesting result in this subgroup was the positive correlation FA had with CRP in BA25. It was originally conceptualized that non-chronic patients hadn't experienced inflammation for long enough to damage their white matter tracts. However, the dynamic gliogenic and gliotoxic effect of inflammation may account for these differences. It is plausible that in the short term, there is WM resiliency to the effects of inflammation—that is the WM compensates and over myelinates in response to the inflammation. However, over time, WM loses its ability to compensate for WM degradation and axonal demyelination results instead.

Taken together, the results of these analyses may inform the inconsistent results across the inflammation and DTI studies of MDD in the literature. While many investigators (Miller et al., 2009) have found elevated inflammatory cytokines cause glutamate excitotoxicity and oligodendroglial death, others indicate that low-grade inflammation may spur neuroplasticity (Goldstein et al. 2016) and increased neurotrophic support for neurons and oligodendroglia. Gliogenesis and gliotoxicity are dynamic and complicated processes that may both be affected

by low-grade inflammation and potentially the duration of illness. The opposite effect of inflammation in the chronic and non-chronic patients might account for the absence of significant effects found in the sample as a whole (Haroon et al. 2016). Another possible reason for variable findings in the literature is that DTI methods and techniques used in previous papers are no longer up to current standards. Differences in the presence of depression subgroups across studies, such as treatment-resistant, late-onset, early trauma exposure, and familial, could also contribute to discrepant results. Furthermore, the impact of depression severity and chronicity that emerged from on our WM analyses (regardless of inflammation), reveals that these key clinical variables must be controlled for or analyzed using stratification for DTI studies of MDD. Finally, another potential confound across studies is the current and lasting impact on WM from MDD treatment interventions. To date, no studies have examined changes in WM integrity after treatment with antidepressant medication.

There are several strengths to this study that increase confidence in the results. First, in terms of sample size, this is the largest study to date exploring the correlation of CRP or IL6 inflammatory cytokines with DTI indexes in MDD patients and also the largest study of DTI in MDD overall. These findings therefore are more likely to better estimate the relationship between clinical variables and inflammatory cytokines and WM tracts in MDD. Second, we undertook state of the art efforts to maximize the quality of the DTI data. A phase reversal distortion correction was used to the DTI images to reduce eddy, motion, and frontal distortion/artifacts because of sinuses (Huang et al, 2008; Wu et al, 2008). Very few other published studies have used this method, which has now become standard corrective practice. Third, we utilized the most corroborated and advanced analytical method to reduce possible errors for DTI imaging acquisition and TBSS. As aforementioned, non-diffusion (b = 0) images

were acquired during each scan session, which were manually averaged to improve signal to noise ratio. Fourth, sixty non-collinear diffusion directions and isotropic voxels were used to improve angular resolution and data integrity, far more than previously used in DTI studies. Fifth, well-established diffusion analysis methods were used to calculate FA value, and a rigorous statistical threshold was applied. Finally, the PReDICT sample was very well characterized clinically, and the treatment-naïve aspect of the patients eliminated any effects on WM that may have arisen from prior treatments.

A limitation to the current study includes lack of standardization around timing of the blood draw for IL-6 and CRP and the lack of repeated measures of inflammation over time to evaluate the persistent elevation of these inflammatory markers. The study also lacked healthy controls against which the DTI and inflammation measures could be compared.

In conclusion, this study identified several potentially important findings that can inform our understanding of the role of WM integrity in the pathophysiology of MDD. In particular, the impact of chronicity, and potentially the added effect of inflammation among chronically ill patients, is a highly salient issue that will warrant further research. The impact of WM integrity on treatment outcomes also needs to be explored, The PReDICT sample randomized patients to receive either CBT or pharmacotherapy, so the WM results reported here will be tested for their impact on treatment outcomes. Ultimately, this work could help inform treatment selection decisions for individual patients and thereby improve outcomes for MDD patients entering treatment.

Tables

Table 1: Demographics for Sample Set

-	All (n =125)	Female	Male	p value
Age (Years)	38.52 ±11.3	37.14 ±10.7	40.65 ±11.3	0.098
Gender	125	49	76	-
HDRS-17	19.37 ±3.7	20.01 ±3.96	18.31 ±3.16	**0.013**
CRP (mg/L)	3.06 ±6.38	3.09 ±5.21	3.01 ±7.81	0.952
IL-6 (pg/ml)	1.51 ±1.45	1.69 ±1.44	1.23 ±1.43	0.086
Chronic Episode	35.2%	39.5%	28.6%	0.213
FHx MDD	41.6%	44.7%	36.7%	0.376
FHx Bipolar	18.4%	22.4%	12.2%	0.154

** Bolded p values indicate significant differences between male and female groups.

HDRS-17 = Hamilton Depression Rating Scale
FHx = Family History

Table 2: DTI Results Summary

Variable	FA finding	AD Finding	RD Finding	MD Finding
Age	Global		Global	
Depression Severity	(-)*		(+)*	
Chronicity			*	
Inflammation				
Male /Female				
Chronic Group: Inflammation	(-)*			
Nonchronic Group: Inflammation	(+)*	(-)*		

Description: This table summarizes the significant findings amongst the 7 DTI analyses performed in this study. If a generalized linear model was used, the directionality of the association was specified using (+/-) to specify a positive/negative correlation, respectively. Asterisks (*) represent significant findings.

Figures

Figure 1: Diagram of Tensor

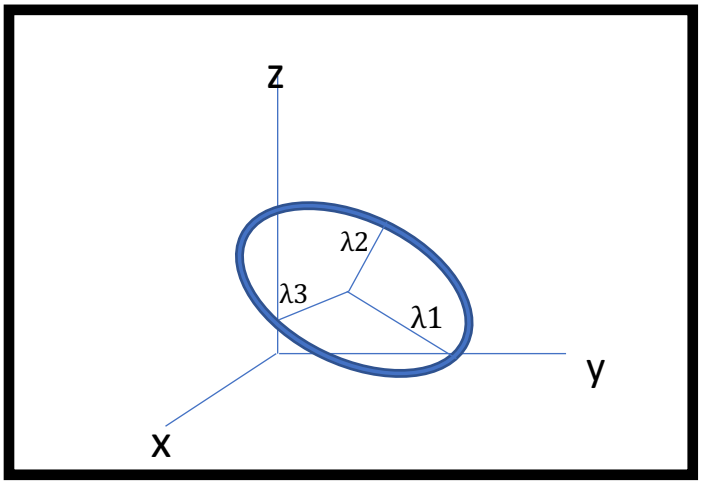
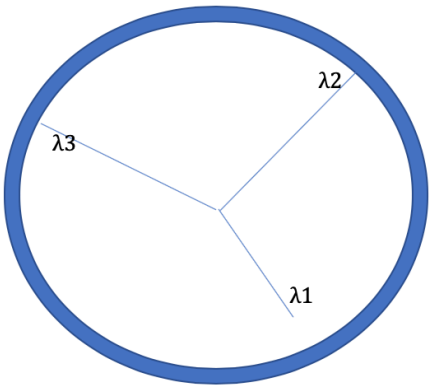
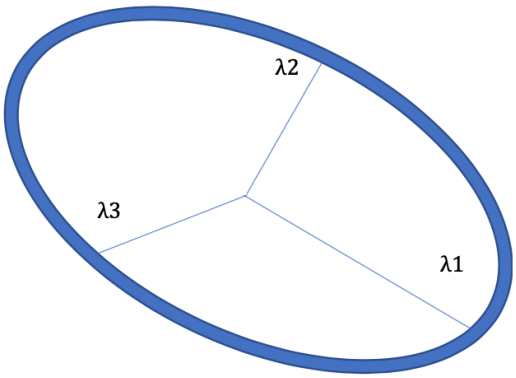


Figure 2: Diagram of Anisotropic and Isotropic Tensors



Isotropic



Anisotropic

Figure 3: Consort Chart- Schematic for preprocessing

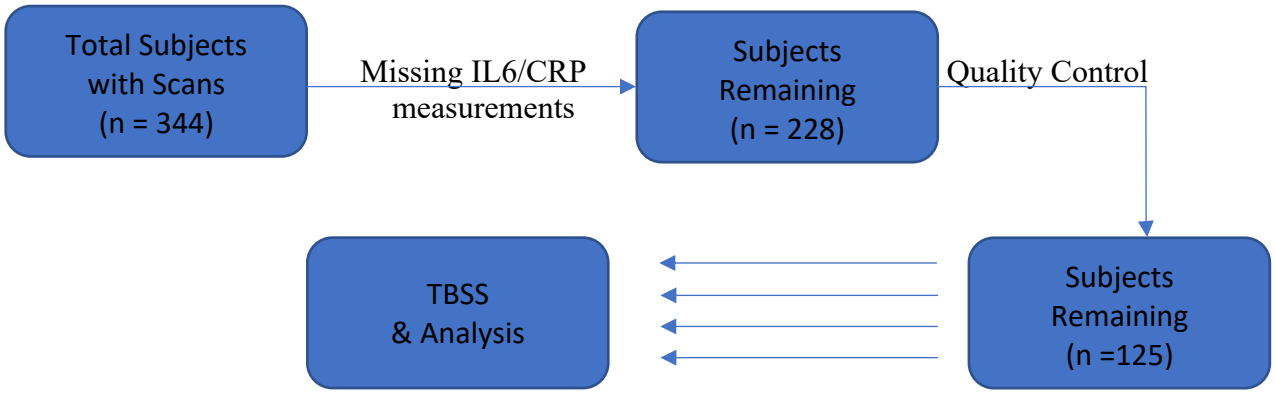
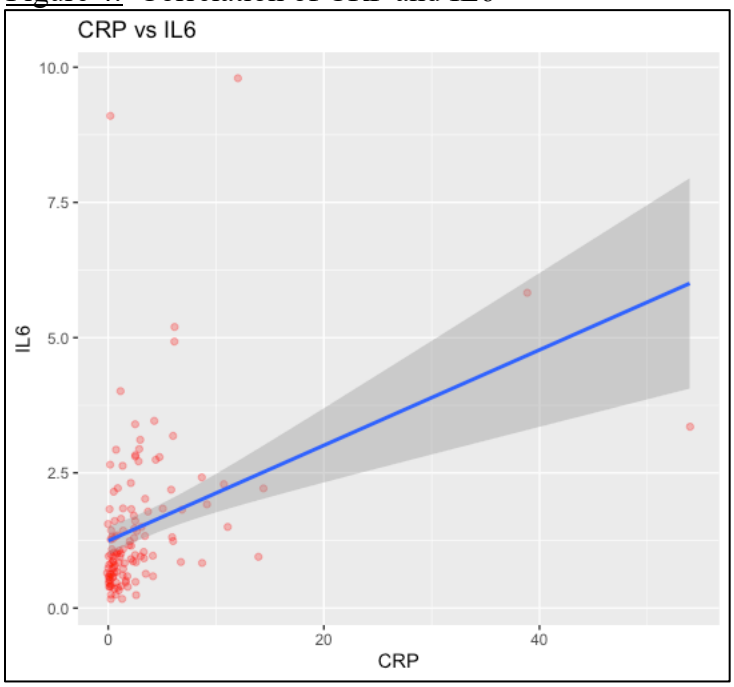


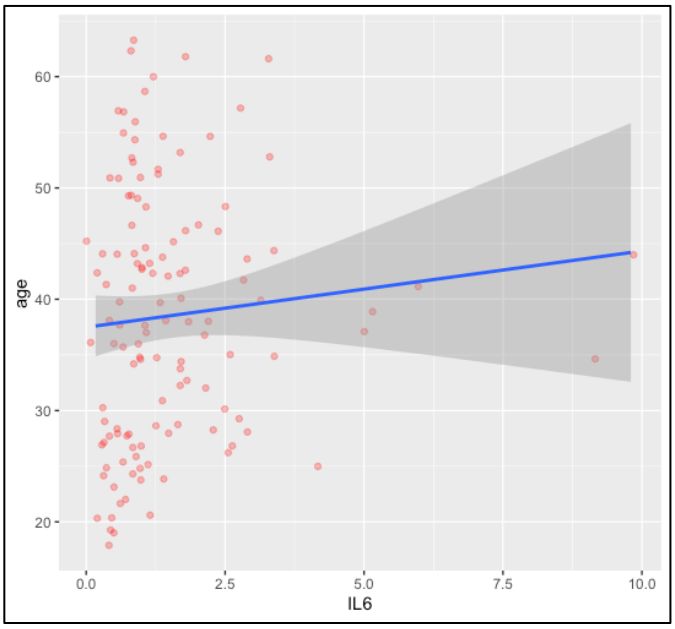
Figure 4: Correlation of CRP and IL6



(R = .38, **p = 1 x 10^{-5}**)

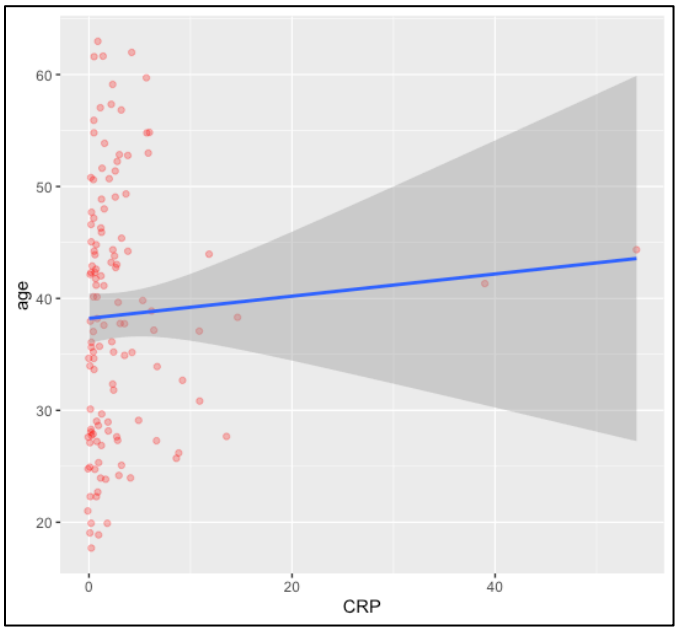
CRP measured in (mg/L)
IL-6 measured in (pg/ml)

Figure 5: Correlation of IL6 and Age



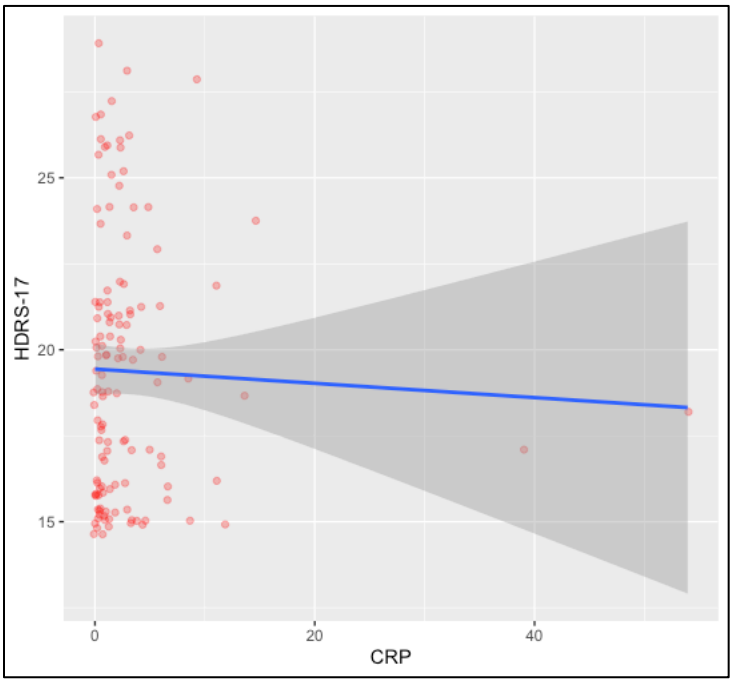
(R = .05, p=.535) IL-6 measured in (pg/ml)

Figure 6: Correlation of CRP and Age



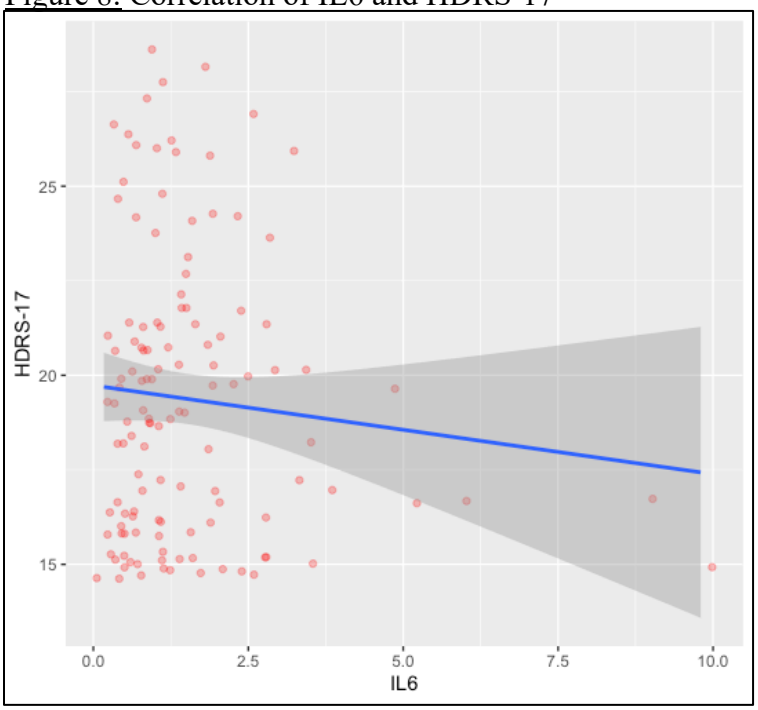
(R =.08, p=.327) CRP measured in (mg/L)

Figure 7: Correlation of CRP and HDRS-17



(R = -0.03, p = 0.697) CRP measured in (mg/L)

Figure 8: Correlation of IL6 and HDRS-17



(R = = -0.09, p = .3119) IL-6 measured in (pg/ml)

Figure 9: FA is significantly lower in Older Patients

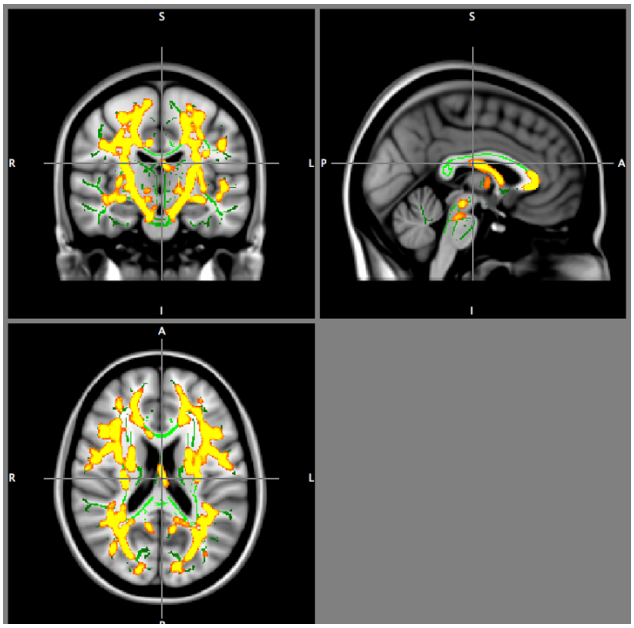


Figure 9. Significant (p <.03) regions of FA comparing Older vs Younger patients. Significant regions (yellow) have lower values in older patients when compared to younger

Figure 10: RD is significantly higher in Older Patients

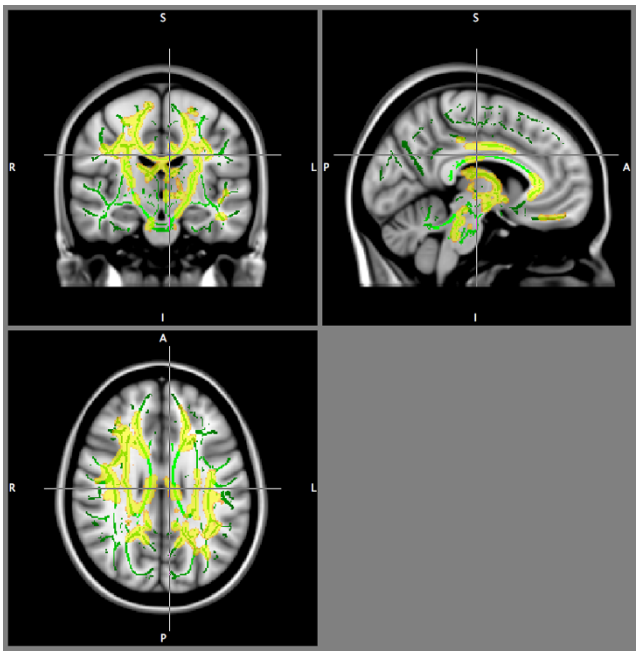


Figure 10. Significant (p < 0.03) regions of RD comparing Older vs Younger patients. Significant regions (yellow) have higher values in older patients when compared to younger.

Figure 11: Significant negative correlation between FA and HDRS-17

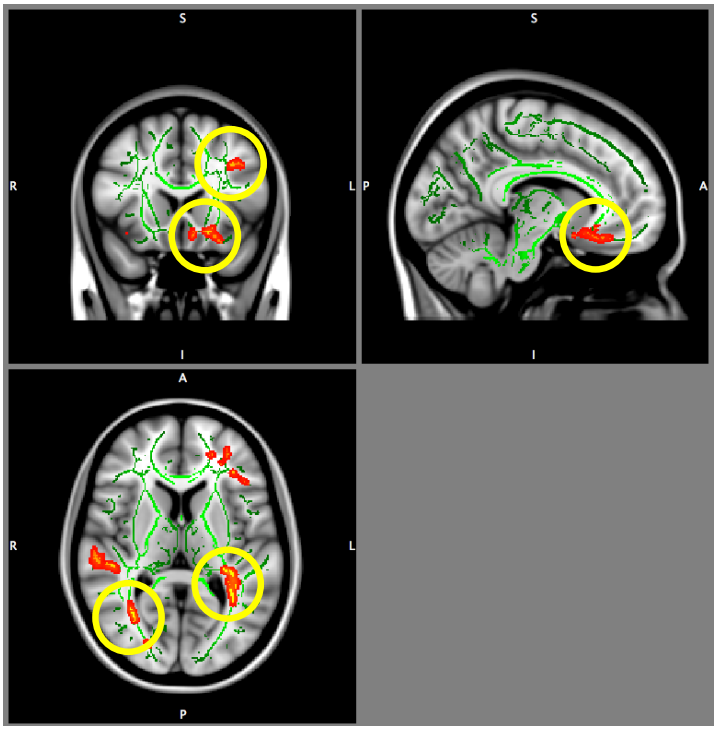


Figure 11. Significant (p < .02) areas (Red/Yellow) indicate a negative correlation of FA with HDRS-17 scores. A more yellow coloring indicates a higher degree of significance. Green represents voxel skeletons of important white matter regions in the brain.

Figure 12: Significant positive correlation between RD and HDRS-17

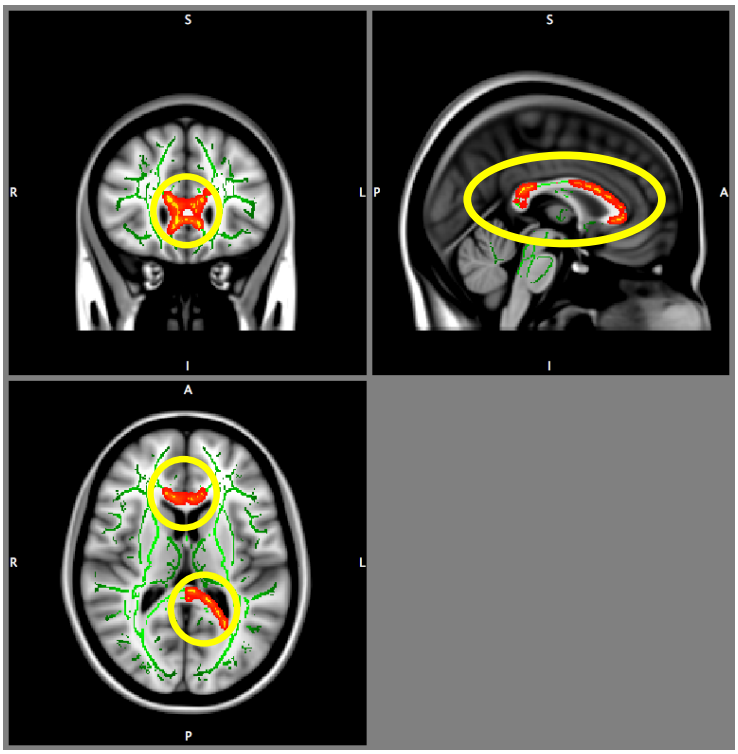


Figure 12: Significant (p <.03) areas (Red/Yellow) indicate regions with a significant positive correlation with HDRS-17 depression severity measures. A more yellow coloring indicates a higher degree of significance. Green represents voxel skeletons of important white matter regions in the brain.

Figure 13: Two Sample T-Test between Chronic and Non-Chronic Patients

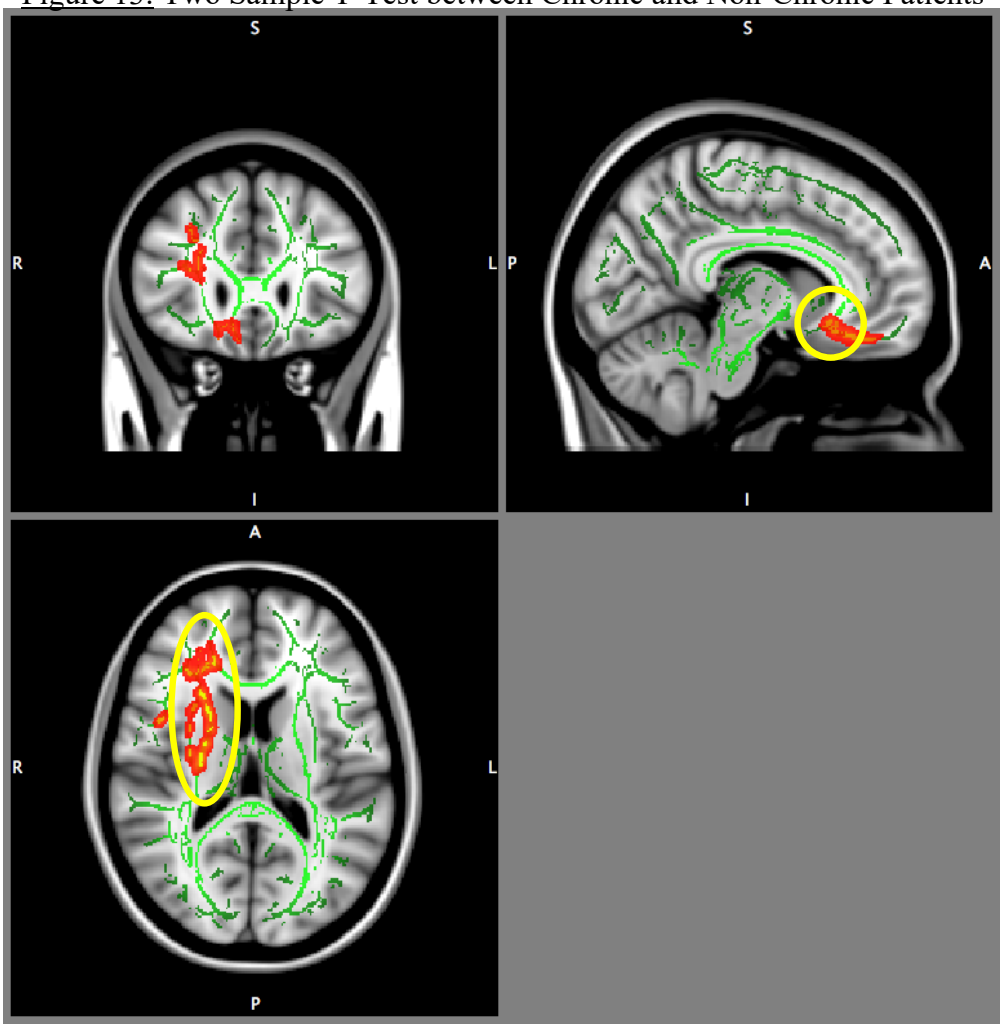


Figure 13. Significant ($p < .04$) are indicated by red/yellow clusters. These regions have higher RD values in chronic patients when compared to nonchronic. A more yellow coloring indicates a higher degree of significance. Green represents voxel skeletons of important white matter regions in the brain.

Figure 14: Negative correlation between CRP and FA within Chronic Patients

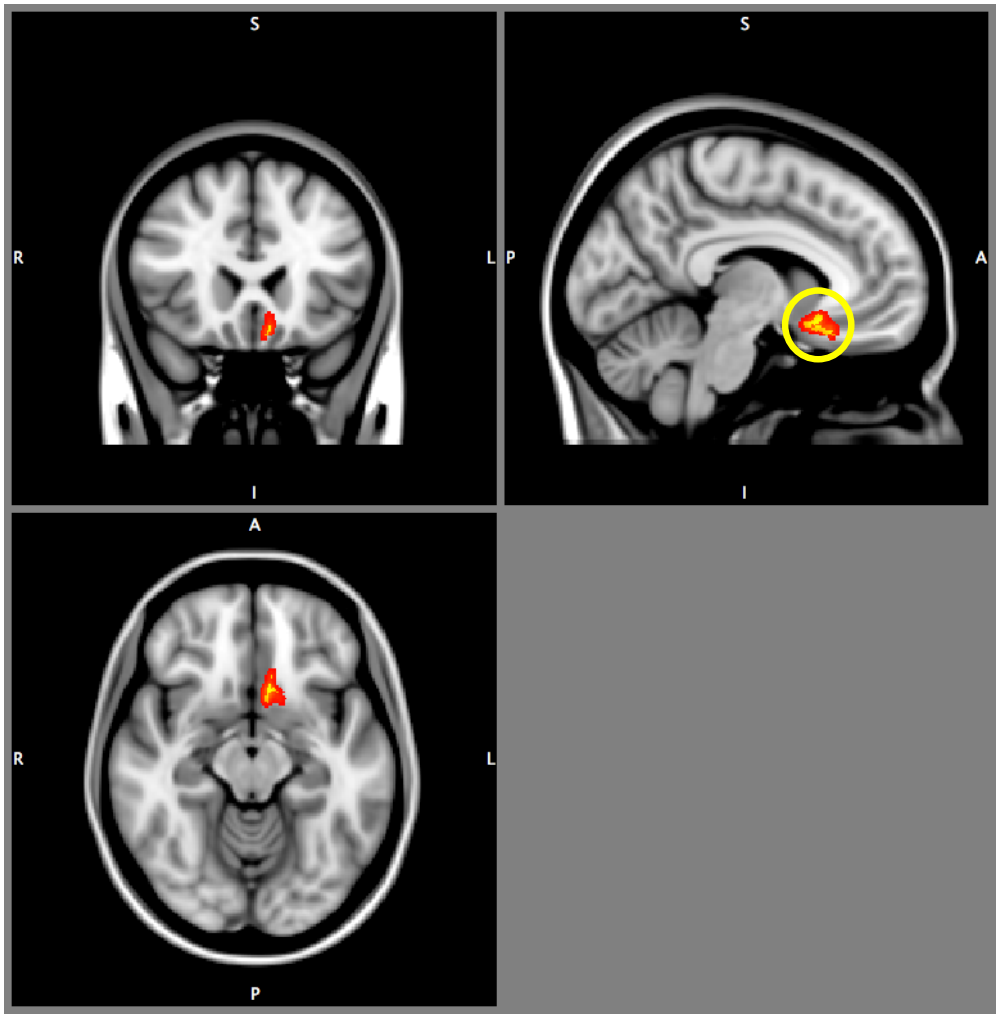


Figure 14. Significant ($p < .05$) areas are indicated by red/yellow regions on the MRI scan. These areas describe a negative relationship between CRP and FA in chronic patients. A more yellow coloring indicates a higher degree of significance.

Figure 15: Significant negative correlation between AD and IL6 within Non-Chronic Patients

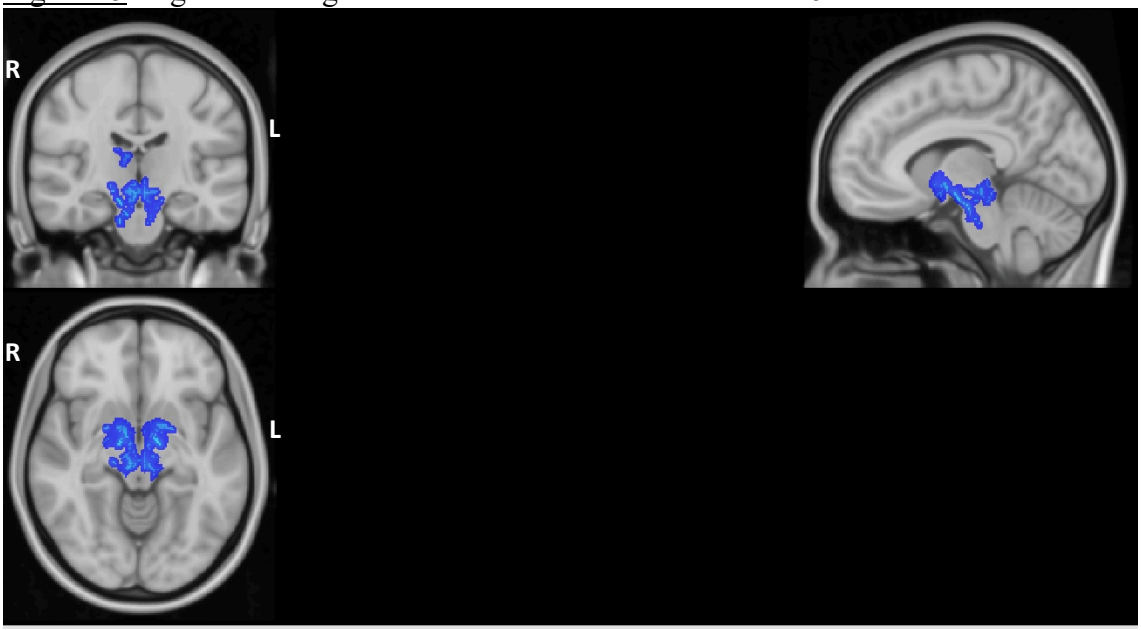


Figure 15. Significant (p < .05) are indicated by blue/light-blue clusters. These regions have a negative correlation with IL6 amongst non-chronic patients. A lighter blue coloring indicates a higher degree of significance.

Figure 16: Positive correlation between FA and CRP in Non-Chronic Patients

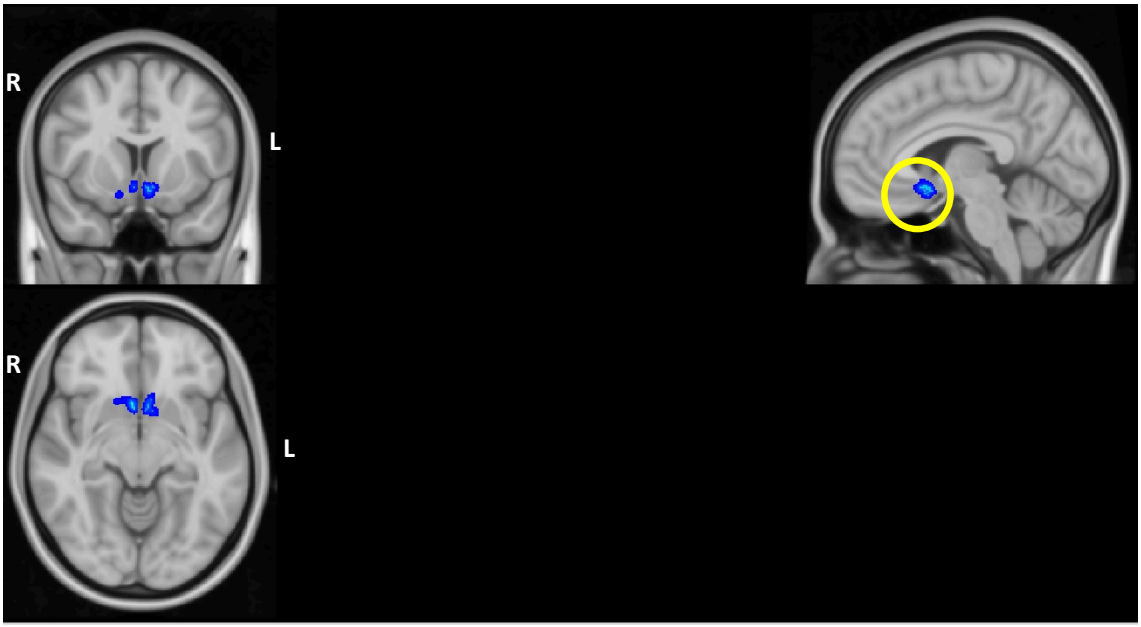


Figure 16. Significant (p < .05) are indicated by blue/light-blue clusters. These regions have a positive correlation with CRP amongst non-chronic patients. A lighter blue coloring indicates a higher degree of significance.

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