# Microstructural Correlates of Resilience against Major Depressive Disorder: Epigenetic Mechanisms?

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Word count: Introductory paragraph: 194, text: 1530, methods: 1079, 1 Tables, 3 Figures, 2 Supplementary Tables, 1 Supplementary Figure

## Introductory Paragraph:

Mental disorders are a major cause of long-term disability and are a direct cause of mortality, with approximately 800.000 individuals dying from suicide every year worldwide - a high proportion of them related to major depressive disorder (MDD) [1]. Healthy relatives of patients with major depressive disorder (MDD) are at risk to develop the disease. This higher vulnerability is associated with structural [2-4] and functional brain changes [5]. However, we found using high angular resolution diffusion imaging (*HARDI*) with 61 diffusion directions that neuron tracts between frontal cortices and limbic as well as temporal and parietal brain regions are characterized by better diffusion coefficients in unaffected relatives (UHR), who managed to stay healthy, compared to healthy volunteers without any family history for a psychiatric disease (HC). Moreover, those UHR with stronger fibre connections better managed incidences of adversity in early life without later developing depression, while in HC axonal connections were found to be decreased when they had early-life adversity. Altogether these findings indicate the presence of stronger neural fibre connections in UHR, which seem to be associated with resilience against environmental stressors, which we suggest occur through epigenetic mechanisms.

## Introduction:

Stress led to depressive-like states accompanied by atrophy and loss of neurons in the adult hippocampus in experimental studies [6]. These effects of stress seem to depend on individual characteristics since initial vulnerability of a mouse to social defeat stress seems to be determined by its basal concentration of transcription factors (ΔFosB (Fos family transcription factor). Moreover, susceptible versus resilient responses to that stress are associated with the degree of transcription factor ΔFosB induction in response to stress [7]. Recently, we found gene-environment interactions on brain structure in patients with MDD. Hippocampal volumes from patients with MDD carrying the genetic risk-allele (short allele) of the serotonin transporter polymorphism (SLC6A4, 5-HTTLPR) were smaller when they had a history of emotional neglect compared to patients who only had the genetic or the emotional neglect risk factor [8].

Environmental factors like stress do not only change the function of neural cells, they also impact epigenetic patterns [9]. Suicide victims with a history of childhood abuse were observed to have decreased levels of glucocorticoid receptor mRNA and increased cytosine methylation of a neuron-specific glucocorticoid receptor (NR3C1) promoter in the postmortem hippocampus compared to either suicide victims with no childhood abuse or controls, suggesting a common effect of parental care on the epigenetic regulation of hippocampal glucocorticoid receptors [9,10]. The brain`s ability to adapt suggests that it might be possible to increase resilience under positive conditions protecting an indiviudal from becoming depressed.

Diffusion tensor imaging (DTI) is a significant step forward for characterizing microstructural changes or differences, since heretofore studies of white matter bundles were restricted to post-mortem dissection or section-by-section evaluation by *in vivo* imaging. DTI may be used to map and characterize the three-dimensional diffusion of water as a function of spatial location [11]. With DTI a significant reduction has been found in white matter fractional anisotropy (FA) in the dorsolateral prefrontal cortex and in widespread regions of the frontal and temporal lobes [12-16] as well in the left sagittal stratum [17] of patients with MDD compared to healthy controls indicating microstructural white matter alterations in MDD. The aim of the present study was to investigate microstructural changes in white matter tracts in a sample of unaffected healthy relatives (UHR) of patients with MDD at familial risk to develop the disease, but who managed to stay well, compared to healthy subjects (HC) without any familial risk for psychiatric diseases. Our hypothesis was first that decreased fractional anisotropy associated with vulnerability and increased fractional anisotropy associated with resilience will be detected in unaffected healthy 1$^{st}$ degree relatives of patients with MDD

compared to healthy controls. The second objective was to investigate whether early-life adversity interacts with group differences and whether this might add to findings from experimental studies showing the importance of epigenetic mechanisms.

**Results:**

UHR and HC did not differ in demographic variables or in early life adversity ratings. Sub threshold depression scores were significantly higher in UHR compared to HC reflecting the familial risk for developing MDD, whereby all values were still within the normal range (Supplementary Table 1). Childhood adversity was not associated with higher depression scores in UHR ($Z=-0.6$, $p=0.61$) or HC ($Z=-0.9$, $p=0.41$). In DTI significantly larger FA values were detected in UHR compared to HC subjects in the posterior body of the corpus callosum, left inferior fronto-occipital fasciculus, left superior longitudinal fasciculus, left external capsule, left thalamus and left anterior thalamic radiation (Table 1, Supplementary Figure 1). These changes can be related to smaller minor eigenvalues ($\lambda 3$) that were found in UHR subjects compared to HC subjects even more prominent in the same regions on both hemispheres and in the right uncinate fasciculus (Figure 1, Supplementary Table 2) indicative for higher myelination. Mean or radial diffusivity and the other eigenvalues ($\lambda 1$, $\lambda 2$) did not differ significantly between groups.

When early life adversity was taken into consideration to test for environment-genetic interactions, a significant two-way interaction between group (UHR < HC) and childhood stress (no childhood stress > childhood stress) was detected for FA most prominent in the right inferior fronto-occipital fasciculus, but also with extensions to the right uncinate fasciculus, right superior longitudinal fasciculus as well as in the splenium and body of the corpus callosum. This interaction indicated significant areas with smaller FA when HC subjects suffered childhood stress compared to when they did not have childhood stress and at the same time larger FA in UHR subjects suffering from childhood stress compared to those without childhood stress (Figure 2A, B). Post hoc analysis in these clusters proved that the UHR and childhood stress group had larger FA than the UHR without childhood stress group. Those HC that were grouped as having childhood stress on the other hand showed smaller FA compared to those HC without childhood stress. Extracting the FA values from the seed voxel in each region gave the opportunity to correlate these FA with childhood stress scores. Again childhood stress correlated positively with FA in UHR (Figure 2C) and negative in HC (Figure 2D). No differences were seen in depression severity between UHR with and without early life adversity, although there was a positive correlation between

depression severity and FA values in the whole group of subjects reflecting the fact that UHR had significant higher FA values and higher depression scores compared to HC.

In order to further explore these differences deterministic tractography was performed. The interaction was confirmed for the right inferior fronto-occipital fasciculus ($F=18.5$, $df=1,39$, $p<0.001$) with larger tract volumes in UHR with childhood stress compared to HC with childhood stress ($t=2.3$, $df=1,21$, $p=0.03$) and smaller tract volumes in UHR without childhood stress compared to HC without childhood stress ($t=3.4$, $df=1,20$, $p=0.003$) (Figure 3). Larger mean FA values were detected in the right inferior fronto-occipital fasciculus ($F=4.0$,$df=1,39$, $p=0.05$) and higher variability of FA was found in the right uncinate fasciculus ($F=6.1$,$df=1,39$, $p=0.018$) in UHR compared to HC.

**Discussion:**

Larger FA and smaller minor eigenvalues λ3 were found for the first time in UHR who managed to stay healthy although they have a familial risk to develop depression compared to unaffected healthy subjects without any family history for psychiatric diseases (HC). The accordance with the pattern of changes in the present study leads to the conclusion that the differences between UHR and HC might result during neurodevelopment for several reasons: 1. Increase in FA and decrease in radial diffusivity with age were detected in children and adolescents [18]. 2. Decrease in eigenvalue λ3 as part of the radial diffusivity component was found to be associated with myelination [19-22].

Interestingly, the increased FA values in fibre bundles connecting prefrontal and orbitofrontal cortices with limbic, temporal, and parietal regions are associated with executive functions, cognitive control and emotion regulation and are in line with previous research showing larger right dorsomedial prefrontal cortex volumes in unaffected relatives of patients with MDD compared to healthy subjects without any family history for a psychiatric disease [2]. These findings might indicate that UHR who did not become depressed, while more likely to carry a high genetic risk, may have some neurobiological characteristics that are associated with increased resilience. Hypothetically UHR subjects developed strategies to stay healthy and to regulate their affects resulting in enriched connections. This brain` s ability to adapt to certain conditions is impressively demonstrated by the fact that learning and physical exercise results in improved plasticity and increased cortical volumes: Wheel running in rodents resulted in enhanced hippocampal neurogenesis [23], improved synaptic plasticity [24,25] and increased spine density [26]. Moreover, exercise and learning caused structural gray matter increases [27,28]. Increased FA and reduced axial diffusivity as shown in the present study are also associated with better cognitive functions like executive functioning, working memory and attention processing [29].

A timely concept is whether epigenetic mechanisms driven by environmental stressors might have a role in preparing certain biological changes for the offspring in advance, so that those might be able to handle similar stressors for example epigenetic mechanisms for resilience. With respect to the second objective we found interactions between early-life adversity and differences between UHR and HC most prominent in the right inferior fronto-occipital fasciculus. FA values and tract volumes were larger in UHR subjects suffering childhood stress compared to those without childhood stress suggesting that those UHR with stronger fibre connections managed more early-life adversity without becoming more depressed than UHR subjects with less fibre connections. Epigenetic processes might mediate the effects of the social environment during childhood on gene expression and stable epigenetic marks such as DNA methylation might then persist into adulthood, might even be passed to the

next generation and might influence vulnerability for psychopathology [9]. Recently it has been shown that the glucocorticoid receptor gene is hypermethylated among suicide victims with a history of abuse in childhood, but not among controls or suicide victims with a negative history of childhood abuse [10]. The present findings again show the importance of interactions between genetic (familial) and childhood stress factors in line with our previous study on the interaction between stress and genetics on hippocampal volumes in MDD [30]. The stronger fibre connections seem to be associated with resilience and might render subjects more stable against environmental stressors suggestively through epigenetic mechanisms. Understanding these biological underpinnings could contribute to the development of new therapy strategies that are able to increase resilience and prevent depressive episodes.

**Methods:**

**Participants**

The study included 21 unaffected healthy relatives (UHR) of patients currently undergoing treatment for MDD at the mental health services of the Adelaide and Meath Hospital, incorporating the National Children's Hospital, Tallaght, Dublin or mental health services at St. James's Hospital, Dublin and 24 healthy subjects without any family history of psychiatric disease (HC) from the local community recruited via announcements (Table 1). Groups were age and gender matched. Each participant was carefully screened for medical conditions so that neither the HC nor the UHR had a personal history of neurological or psychiatric disorder (Axis I or Axis II), or a history of severe medical illness, head injury or substance abuse. Demographic variables, inclusion and exclusion criteria were assessed using a standardized questionnaire and through a structured interview by a psychiatrist.

**Rating Instruments**

Self and observer rated scales were also filled out for all participants included in the study. The rating scales that were used to comprised: the Hamilton Rating Scale for Depression [31], the Montgomery-Asberg Depression Rating Scale (MADRS) [32], Beck's Depression Inventory (BDI-II) [33], Childhood Trauma Questionnaire (CTQ) [34], and the Structured Clinical Interview for DSM_IV (SCID-II) personality questionnaire. The Childhood Trauma Questionnaire (CTQ) was used to assess childhood stress. This questionnaire is a self-report instrument that assesses five types of childhood maltreatment: emotional, physical, and sexual abuse, and emotional and physical neglect. Subjects rated items about childhood experiences (defined as prior to age 18) on five-point Likert-type scales anchored by "never true" and "very often true". Reliability and validity of the CTQ have been established, including measures of convergent and discriminative validity from structured interviews, stability over time, and corroboration [35] [36].

Written informed consent was obtained from all participants after been given detailed description of the study which was designed and performed in accordance to the ethical standards laid out by the Declaration of Helsinki and was approved by the local ethics committees.

**Diffusion Tensor Imaging (DTI)**

Magnetic resonance images were obtained with a Philips Achieva MRI scanner (Philips Medical Systems, The Netherland  do not think we need all these details) operating at 3 Tesla.  DTI with 61 diffusion directions was obtained (Field Of View (FOV): 200 x 257 x 126 mm, 60 slices, no gap, spatial resolution:  1.8 x 1.8 x 2.1 mm, TR / TE = 12561 / 59 ms, flip angle = 90°, half k-space acquisition was used (half scan factor = 0.68), SENSE parallel imaging factor = 2.5, b-values = 0, 1200 s/mm$^2$, with SPIR fat suppression and dynamic stabilisation in an image acquisition time of 15 min 42 s). Data were analyzed using tractography in Explore DTI [37] and Tract-Based Spatial Statistics (TBSS) implemented in FSL [38].

The underlying physical process of diffusion (by Brownian motion) causes a group of water molecules to move out from a central point, and gradually reach the surface of an ellipsoid if the medium is anisotropic (it would be the surface of a sphere for an isotropic medium). The ellipsoid itself has a principal long axis and then two more small axes that describe its width and depth. All three of these are perpendicular to each other and cross at the center point of the ellipsoid. We call the axes in this setting eigenvectors and the measures of their lengths eigenvalues λ. The diffusivity along the principal axis, $\lambda_1$ is also called the longitudinal diffusivity or the axial diffusivity. The diffusivities in the two minor axes are often averaged to produce a measure of radial diffusivity $(\lambda_2 + \lambda_3)/2$. If we divide this sum by three we have the mean diffusivity. The fractional Anisotropy or FA is the square root of the sum of squares (SRSS) of the diffusivity differences, divided by the SRSS of the diffusivities.

In order to understand the possible relevance of these findings limitations need to be discussed. Tract-based spatial statistics as used here minimize the effects of misalignment and are more robust and sensitive than voxel based analysis [38]. Tract-based spatial statistics, however, are limited to investigating local changes in white matter integrity, and interpreting differences in regions of crossing fibers can be complex [38]. Considerable areas of fiber crossing exist, for example in the centrum semiovale, uncinate fasciculi, and transpontine fibers, which have corresponding low FA and are difficult to investigate, because they would show less likely group differenes. Fewer white matter crossings are seen for example in the corpus callosum [39] and therefore our results on corpus callosum seem not to be influenced much by fiber crossings. Tractography is an advantage with this respect because it allows to track a fibre bundle throughout the brain and then to explore its FA, eigenvalues, number and volume of tracts [37]. Interpretation of DTI data is complicated by the sensitivity of the diffusion tensor, and the anisotropy in particular, to a broad spectrum of other factors, including image noise (both thermal and physiologic) artifacts (e.g.,

misregistration of DW images from eddy currents or head motion) [40,41], partial volume averaging between tissues in large voxels (e.g., signal mixing of gray matter, WM and CSF) [42]. However, noise and artifacts would more likely result in non-significant findings, and thus the findings from the present study which are corrected for multiple comparisons can be seen to be strong.

**Statistics**

Differences in demographic variables were tested using Student` s T-test, Chi-square test for gender distribution and Mann Whitney U test for differences in clinical variables. Using Threshold-Free Cluster Enhancement (TFCE)[43] the statistical threshold was set $p<0.05$ fully corrected for multiple comparisons across space to find differences between UHR and HC for FA, mean diffusivity (MD), radial diffusivity (RD), axial diffusivity (λ1) and eigenvalues λ2 and λ3. We used also a median split for childhood stress in order to test for interactions between childhood stress and diagnosis ($p<0.05$ fully corrected for multiple comparisons across space). Fractional anisotropy (FA) values from significant clusters were extracted for further regression analysis on the influence of age and depression scores. For the analysis of tractography results an ANCOVA design to assess the main and interaction effects of the between-subjects factors group (UHR, HC) and childhood stress (yes, no) using age and gender as covariates.



**Acknowledgement:** The authors do not report any conflicts of interest. Professor Frodl received a Stokes Professorship award from Science Foundation Ireland (SFI) to carry out these investigations.

**Supplementary Results:**

A significant negative correlation was detected between age and FA in the superior longitudinal fasciculus ($r=-0.52, p<0.001$). Age was found to be negatively associated with the FA values, so that age would not explain the increased FA values in UHR patients, because this would have led to smaller FA rather than larger FA in UHR.

Significant positive correlations were detected between severity of depression measured with Hamilton Depression Rating Scale and FA in the anterior body of corpus callosum ($r=0.48$, $p=0.001$), posterior body of corpus callosum ($r=0.34$, $p=0.022$), left anterior thalamic radiation ($r=0.37$, $p=0.013$) and left thalamus ($r=0.38$, $p=0.01$). There was no significant interaction between subgroup (UHR, HC) and depression severity in the regression analysis.

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**Table 1:** Fractional anisotropy (FA) differences between groups. Table shows regions with increased FA values for UHR compared to HC. P-value < 0.05 corrected for multiple testing. x y z coordinates are given in MNI space.

| Region | voxels | p-value | x | y | z |
|---|---|---|---|---|---|
| **Body of corpus callosum** | 261 | 0.039 | 81 | 96 | 96 |
| **Body corpus callosum** | 62 | 0.046 | 101 | 98 | 98 |
| **Anterior thalamic radiation, left** | 158 | 0.041 | 117 | 81 | 93 |
| **Inferior fronto-occipital fasciculus, left** | 95 | 0.045 | 116 | 102 | 71 |
| **Superior longitudinal fasciculus, left** | 71 | 0.045 | 138 | 120 | 93 |
| | | | | | |
| **Thalamus, left** | 22 | 0.049 | 103 | 98 | 70 |
| **external capsule, left** | 20 | 0.049 | 123 | 116 | 78 |

**Figure 1:** Decreased eigenvalue L3 in UHR compared to HC. Significant areas are highlighted in Red-Yellow (corrected for multiple comparisons). Body and splenium of corpus callosum, forcepts minor right, left and right inferior fronto-occipital fasciculus (temporal and parietal part) and superior longitudinal fasciculus (temporal and frontal part). The uncinate fasciculus also showed smaller minor eigenvalue L3 (not shown here). Interestingly, the fibers of the uncinate fasciculus connect the amygdala and the hippocampus with the orbitofrontal cortex [44]. These regions play a role within the network for affect regulation and the processing of social and emotional stimuli [45]. The superior longitudinal fasciculus III connects lateral parts of the inferior parietal lobule with the lateral inferior prefrontal lobe in bidirectional way [46] and plays a role in the frontoparietal circuit involved in working memory, the articulary component of language [46,47] and attentional processes, like spatial biasing [48,49]. Disrupting of any one of these functions may compromise self-regulatory capacities. Posterior fibres of the corpus callosum are associated with executive functions and memory [50]. The dorsolateral and premotor prefrontal cortices are connected to posterior part of the parietal, temporal and occipital lobes as well as the caudal cingulate gyrus via the inferior fronto-occipital fasciculus [51] involved in awareness and executive functions [52].

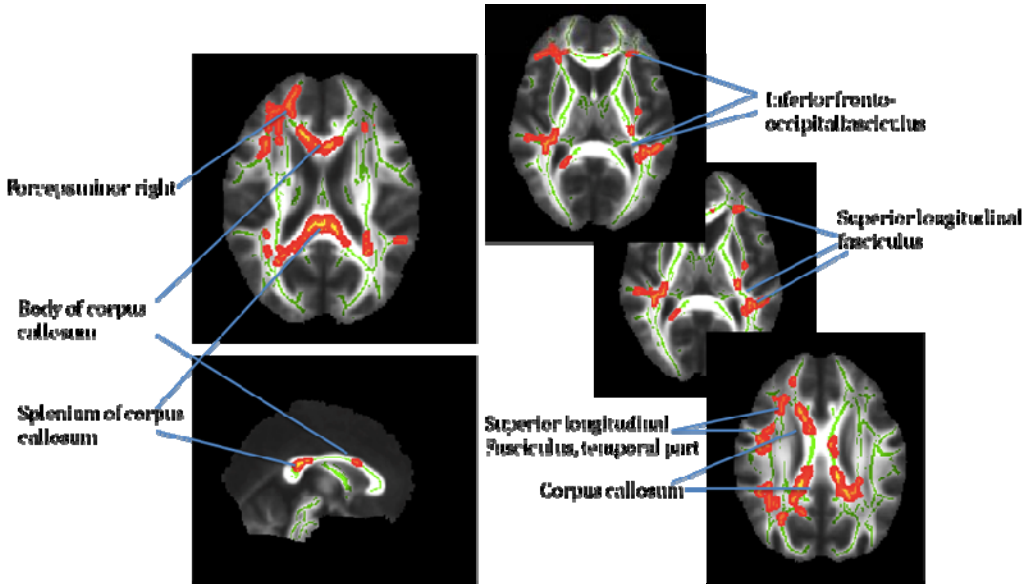


**Figure 2:** Interaction between group (UHR, HC) and childhood stress. Significant areas were in the uncinate fasciculus (UF) (x=68, y=148, z=63), inferior fronto-occipital fasciculus (FOF) (x=58, y=108, z=76) (temporal and frontal part), superior longitudinal fasciculus (SLF) (temporal part) (x=63, y=135, z=97) and the splenium of the corpus callosum (CC) (x=72, y=83, z=99) and are highlighted Red-Yellow (corrected for multiple comparisons) in axial (1. row) and coronal (2. row) slices. UHR subjects had larger FA with more childhood stress, while HC obtained smaller FA with more childhood stress. Correlations between FA in the voxel of the peak coordinate and childhood stress (CTQ) are shown in row 3 for UHR (UF: r=0.47,p=0.03, FOF: r=0.37,p=0.09, SLF: r=0.34,p=0.12, CC: r=0.50,p=0.02) and in row 4 for HC (UF: r=-0.11,p=0.6, FOF: r=-0.57,p=0.004, SLF: r=-0.33,p=0.11, CC: r=-0.39,p=0.06).

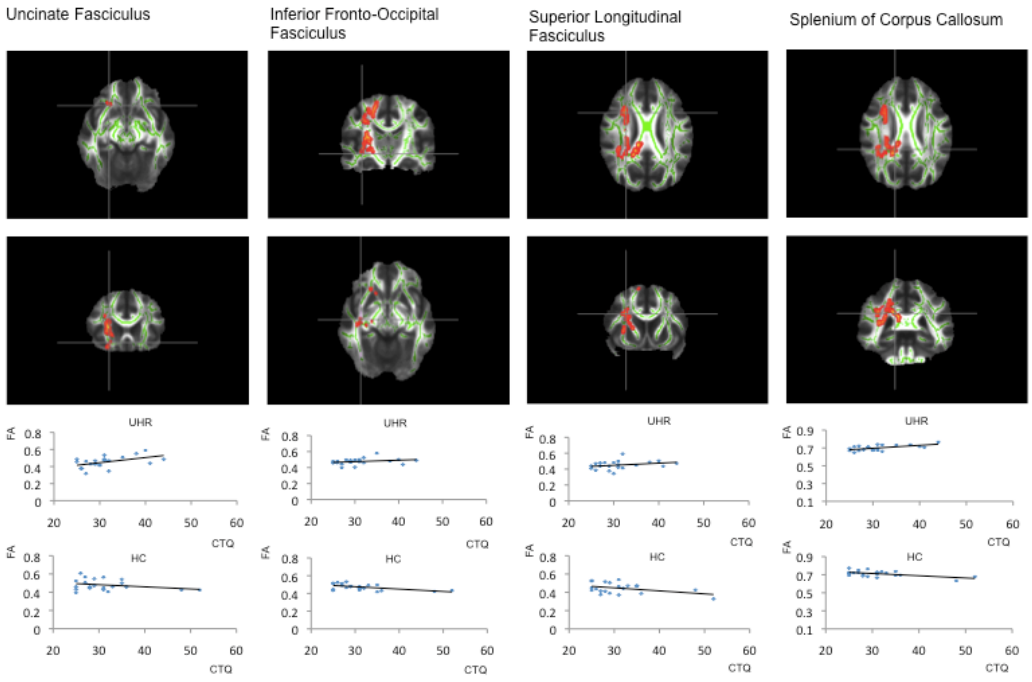
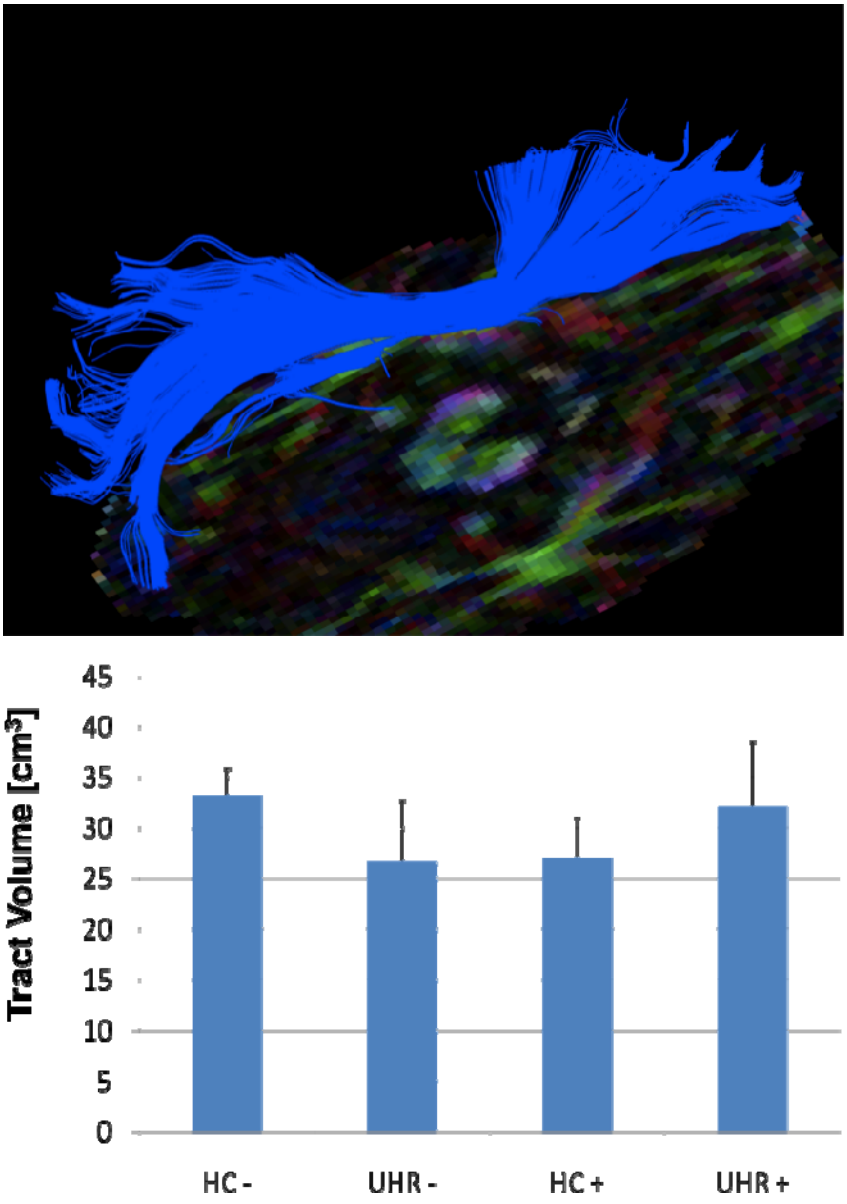


Figure 3: Volume of right inferior fronto-occipital fasciculus in the subjects with and without early life adversity or family history for MDD. An example of the inferior fronto-occipital fasciculus is depicted from one subject in the figure at the top. Mean values and standard deviation for groups are shown in the image below: UHR with childhood stress showed larger tract volumes compared to HC with childhood stress (t=2.3, df=1,21, p=0.03). Tract volumes were smaller in UHR without childhood stress compared to HC without childhood stress (t=3.4, df=1,20, p=0.003). HC with childhood stress had smaller tract volumes compared to those without childhood stress (t=4.6, df=1,22, p<0.001), while UHR with childhood stress had larger tract volumes than those without childhood stress (t=2.0, df=1,19, p=0.06).



**Supplementary Table 1:** Demographic and clinical data of participants (HC: healthy controls; UHR unaffected relatives from patients with MDD). Indicated are the p-values from the t-statistics (chi-square test for gender differences).

| | HC (N=24) | | UHR (N=21) | | **p-value** |
|---|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** | |
| Age (years) | 34.7 | 11.0 | 38.1 | 14.5 | 0.37 |
| Gender* (female/male) | 14/10 | | 13/8 | | 0.81 |
| Weight (kg) | 71.5 | 17.1 | 67.3 | 13.4 | 0.37 |
| 21-item Hamilton Depression scale Score | 1.5 | 1.7 | 4.0 | 3.9 | 0.007 |
| Beck Depression Inventory | 1.5 | 2.1 | 3.7 | 5.7 | 0.089 |
| Childhood stress | 31.3 | 6.8 | 31.3 | 5.4 | 0.98 |
| Emotional neglect | 7.2 | 2.9 | 7.4 | 2.9 | 0.77 |
| Physical neglect | 6.2 | 1.6 | 6.3 | 1.8 | 0.88 |
| Sexual abuse | 5.6 | 1.4 | 5.5 | 1.2 | 0.88 |
| Emotional abuse | 6.5 | 2.0 | 6.3 | 1.9 | 0.77 |
| Physical abuse | 5.8 | 1.7 | 5.7 | 1.4 | 0.87 |

* Chi-square test for gender differences between groups

**Supplementary Table 2:** Eigenvalues λ3 differences between groups. Table shows regions with increased FA values for UHR compared to HC. P-value < 0.05 corrected for multiple testing. x y z coordinates are given in MNI space.

| Region | voxels | p-value | x | y | z |
|---|---|---|---|---|---|
| **Splenium of corpus callosum, extension to forceps major, inferior fronto-occipital fasciculus** | 5168 | 0.025 | 87 | 94 | 94 |
| **Genu corpus callosum, Forceps minor, right** | 437 | 0.044 | 77 | 148 | 93 |
| **Forceps minor, right** | 1195 | 0.041 | 62 | 163 | 92 |
| **Forceps minor, right** | 12 | 0.049 | 77 | 159 | 76 |
| **Forceps minor, left** | 44 | 0.049 | 98 | 159 | 74 |
| **Superior longitudinal fasciculus, right, temporal part** | 471 | 0.045 | 55 | 128 | 105 |
| **SLF right, temporal part** | 158 | 0.046 | 51 | 117 | 99 |
| **Superior longitudinal fasciculus, right, temporal part** | 13 | 0.049 | 53 | 106 | 101 |
| **Superior longitudinal fasciculus, left, temporal part** | 286 | 0.046 | 138 | 89 | 81 |
| **Superior longitudinal fasciculus, left, temporal part** | 250 | 0.046 | 135 | 82 | 72 |
| **Inferior fronto-occipital fasc., right** | 54 | 0.049 | 65 | 159 | 83 |
| **Inferior fronto-occipital fasc., right** | 13 | 0.049 | 64 | 78 | 95 |
| **Inferior fronto-occipital fasc., left** | 722 | 0.037 | 119 | 104 | 70 |
| **Inferior fronto-occipital fasc., left** | 25 | 0.049 | 132 | 92 | 65 |
| **Inferior fronto-occipital fasc., left (frontal)** | 227 | 0.048 | 120 | 159 | 83 |
| **Inferior longitudinal fasc., left** | 120 | 0.048 | 127 | 88 | 79 |
| **Uncinate fasciculus, right** | 26 | 0.049 | 132 | 92 | 65 |
| **External capsule, left** | 20 | 0.049 | 123 | 116 | 78 |

**Supplementary Figure 1:** Increased FA values in the posterior body of the corpus callosum and the superior longitudinal fasciculus in UHR compared to HC. Significant areas are highlighted in Red-Yellow (corrected for multiple comparisons).