Untangling the adolescent internalizing brain: investigations on brain networks in youth with anxious and depressive problems

Roelofs, E.F.


Part 1

2

Exploring the course of adolescent anxiety and depression: associations with white matter tract microstructure

Eline F. Roelofs, Janna Marie Bas-Hoogendam, Steven J.A. van der Werff, Saskia D. Valstar, Nic J.A. van der Wee, Robert R.J.M Vermeiren

Eur Arch Psychiatry Clin Neurosci 2022. Vol 272 Issue 5 Pages 849-858

Abstract

Objective: Cross-sectional Diffusion Tensor Imaging (DTI) studies have reported alterations in white matter (WM) microstructure in adolescents with internalizing psychopathology. Yet, longitudinal studies investigating the course of WM microstructure are lacking. This study explored WM alterations and its relation to clinical symptoms over time in adolescents with internalizing disorders.

Methods: DTI scans were acquired at baseline and after three months in 22 adolescents with clinical depression and comorbid anxiety (INT), and 21 healthy peers (HC) (age: 12 – 18). Tract-based spatial statistics was used for three voxelwise analyses: i) changes in WM microstructure between and within the INT and HC group; ii) associations between changes in symptom severity and changes in WM microstructure within youths with INT; iii) associations between baseline WM parameters with changes in symptom severity within youths with INT.

Results: Data did not reveal changes in WM microstructure between or within groups over three months' time nor associations between changes in WM microstructure and changes in self-reported symptoms (analyses corrected for age, gender and puberty stage). Lower baseline levels of fractional anisotropy (FA) in the right posterior corona radiata (PCR) and right cingulum were associated with a higher decrease of depressive symptoms within the INT group. Post-hoc analysis of additional WM parameters in the significant FA clusters showed that higher levels of baseline mean diffusivity and radial diffusivity in the PCR were associated with a lower decrease in depressive symptoms.

Conclusion: Baseline WM microstructure characteristics were associated with a higher decrease in depressive symptoms over time. These findings increase our understanding of neurobiological mechanisms underlying the course of internalizing disorders in adolescents.

Introduction

Depression is a prevalent mental illness in adolescence and comorbidity with one or more anxiety disorders is high [1, 2]. A substantial proportion of the adolescents diagnosed with depression display more severe symptoms, poorer response to treatment and increased risk of suicidal behaviors. This complex psychopathology is often linked to comorbidity with anxiety disorders [2]. Sadly, recent studies imply that comorbid anxiety disorders are rather the rule than the exception in clinically depressed adolescents as the majority of the adolescent clinical population with depression has one or more comorbid anxiety disorders [2]. Models suggest that complicated interactions between environment, genes, neurobiological characteristics and the timing of developmental stages contribute to the onset and course of internalizing (anxiety and depressive) disorders [3, 4]. Longitudinal studies may shed light on the neurobiological mechanisms involved in the development and course of these impairing disorders and could thus be used to elucidate these interactions. Previous longitudinal studies in at-risk or subthreshold populations have suggested a complex interaction between the course of anxious and depressive symptoms and white matter (WM) neurodevelopmental plasticity, thus underlining the need for longitudinal studies in clinical cohorts [4]. Yet, despite the clear relevance for developing prevention and intervention strategies, only limited longitudinal research into possible neurobiological targets has been conducted in adolescents with internalizing psychopathology (i.e. with clinical anxiety and/or depression).

There are, however, several cross-sectional studies in clinical adolescent anxiety and depression revealing structural and functional abnormalities in neural networks that subserve affective processing, such as abnormalities in the corticolimbic circuitry [3]. Main components of this network include the amygdala, insula, hippocampus, anterior cingulate cortex (ACC) and prefrontal cortex (PFC), which regulate the experience, expression and evaluation of emotions [3]. Alterations in emotion-processing networks have been reported in studies in adolescents with anxiety or depression such as functional and structural changes in amygdalar and ACC networks. For example, impaired amygdala habituation and decreased ACC volume have been reported previously [5, 6]. To our knowledge, few DTI studies in adolescents with clinical depression have been conducted. These studies have reported lower fractional anisotropy (FA), a general indicator of WM tract microstructure, in adolescents with depression compared to healthy peers in WM tracts such as the corpus callosum, cingulum, inferior fronto-occipital fasciculi (IFOF) and uncinate fasciculus (UF) [7-10]. In addition, only one DTI study has been conducted in adolescents diagnosed with an anxiety disorder. Liao *et al.* [11] reported lower FA in adolescents with Generalized Anxiety Disorder (GAD) compared to healthy peers in among others the IFOF, UF and corona radiata. We refer the reader to Supplemental Tables S1a and S1b for an overview of previous literature. The UF, corpus callosum, IFOF and cingulum are thought to be involved in regulation and communication within and between regions of the corticolimbic network [12, 13]. Interestingly, these results seem to show overlapping patterns compared to adult depression and anxiety, although studies in the adolescent population are still few [5, 6]. Considering the above, alterations in functional and

structural regions involved in the corticolimbic network are likely to play a role in adolescent anxiety and depression and could be targets for further investigation.

In addition to these cross-sectional studies, a few longitudinal, non-DTI studies have been conducted in adolescents with internalizing psychopathology. Resting-state functional connectivity (RSFC) studies in adolescent depression have shown alterations over time in RSFC within and between regions involved in emotion processing, such as the amygdala and insula, and cognitive controlling regions such as the ACC and prefrontal cortex. Importantly, baseline and longitudinal changes in RSFC were associated with changes in depression severity [14-16]. Furthermore, structural and functional alterations in the insula and structural changes in lateral and medial prefrontal regions have been associated with future onset and illness course in adolescents at-risk for depression or diagnosed with subthreshold depression [4]. Therefore, baseline characteristics are likely to be associated with the development and course of the disorder. However, longitudinal DTI studies in clinical adolescents with depression and comorbid anxiety are lacking.

The *Emotional Pathways' Imaging Study in Clinical Adolescents* (EPISCA) study is a longitudinal naturalistic study designed to investigate neurobiological mechanisms related to emotion processing and regulation in a clinical cohort of adolescents with stress-related psychopathology. Measurements were taken at baseline, three months and six months. The study consisted of three groups: adolescents with internalizing (depressive and anxiety) disorders, adolescents with trauma disorders and a healthy control group. Results of the neuroimaging measurements at baseline have been previously reported [9, 17-20]. The present work explored WM microstructure in adolescents with depression and anxiety and a control group of healthy peers over a three-month period, in order to gain more insight in longitudinal changes in WM microstructure involved in a clinically representative population of adolescents with internalizing psychopathologies.

In addition, we focused on the within-subject relationship between (baseline) WM microstructure and clinical symptoms over time within the internalizing group. Our main parameter of interest was FA. We used other DTI parameters, being axial diffusivity (AD), mean diffusivity (MD) and radial diffusivity (RD), to gain more insight into characteristics of underlying WM microstructure involved in adolescent anxiety and depression. DTI uses tensors to model diffusivity of water molecules across the brain. These tensors consist of three main eigenvalues (λ_1, λ_2 and λ_3) which in turn can be used to calculate the four most commonly used characteristics of WM microstructure: fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD), and mean diffusivity (MD) [21]. FA provides a relative difference between the largest eigenvalue as compared to the others, reflecting the tendency of water molecules to diffuse in one direction as opposed to all others and could therefore be described as a general indicator for WM microstructure (e.g. myelin thickness, membrane integrity) [21, 22]. AD is defined as the first eigenvalue (l_1) and reflects water diffusion along the principal direction of the fiber, displaying fiber bundle coherence and axonal integrity [23]. RD is defined as the average of the second and third eigenvalue (λ_2 and λ_3) and reflects water diffusion perpendicular to the principal direction of the fiber, thus being more

indicative of the level of myelinization [24]. MD is defined as the average of the three eigenvalues and hence reflects average water diffusion in all directions within a fiber, thus putatively reflective of a degree of myelination [25]. In general, decreased FA is coupled with decreased AD and / or increased RD and MD and vice versa [21, 26].

Based on previous cross-sectional studies of white matter in anxious or depressed adolescents compared to healthy controls and longitudinal studies in at-risk groups [4, 8-11, 27], we expected different FA changes over time in the clinical group compared to healthy peers in WM tracts previously implied in emotion processing, such as the cingulum, UF and corpus callosum. Directionality of these changes will be investigated exploratory as results of previous literature are few and heterogenous.

Methods

Participants

Participants took part in the *Emotional Pathways' Imaging Study in Clinical Adolescents* (EPISCA) study, and were examined three times in a six-month interval between January 2010 to August 2012 [28]. In the present study, only data from two groups of adolescents between 12 and 18 years old were included from the first (baseline) and second visit (three months after baseline), as loss-to-follow-up was too great after six months (loss-to-follow-up after three months: 16%, loss-to-follow-up after six months: 24%).

The first group consisted of adolescents with clinical depression and comorbid anxiety disorders (n = 30; internalizing disorders, hereafter referred to as 'INT'). These participants were recruited in outpatient departments of two child-and-adolescent psychiatric clinics and included in the study before start of care-as-usual (see Supplemental Table S2 for more information about received treatments). They (i) were diagnosed with clinical depression and at least one clinical anxiety disorder as assessed by categorical measures of DSM-IV depressive or anxiety disorders, (ii) were being referred for cognitive behavioral therapy at an out-patient care unit, and (iii) recent change in antidepressant treatment.

The second group consisted of healthy control peers (n = 32, HC), who were recruited through local advertisements. They had (i) no current or past DSM-IV diagnoses of Axis I and/or Axis II disorders, (ii) no clinical scores on validated mood and behavioral questionnaires, (iii) no history of traumatic experiences, and (iv) no current psychotherapeutic and/or psychopharmacological intervention of any kind.

Exclusion criteria for both groups were: (i) a primary DSM-IV diagnosis of attention deficit hyperactivity disorder, oppositional defiant disorder, conduct disorder, pervasive developmental disorders, posttraumatic stress disorder, Tourette's syndrome, obsessive–compulsive disorder, bipolar disorder, and psychotic disorders, (ii) current use of psychotropic medication, (iii) current substance abuse, (iv) a history of neurological disorders or severe head injury, (v) age < 12 or > 21 years, (vi) pregnancy, (vii) left-handedness, (viii) IQ score < 80, as measured by either the Wechsler Intelligence Scale for Children [29]

(WISC) or the Wechsler Adult Intelligence Scale [30] (WAIS), and (ix) general MRI contraindications (e.g. metal implants, claustrophobia).

At the first visit, participants underwent clinical assessment by a child and adolescent psychiatrist. Afterwards, the Anxiety Disorders Interview Schedule [31] (ADIS) was performed to obtain DSM-IV-based classifications of anxiety and depressive disorders. To assess severity of anxious and depressive symptoms, additional self-report questionnaires were completed at each visit, including the Children's Depression Inventory [32] (CDI) and the Revised Child Anxiety and Depression Scale [33] (RCADS). More specifically, total scores on the CDI were used to assess depressive symptoms and total t-scores on the anxiety subscale for the RCADS to assess anxiety symptoms. Clinical scores were defined as ≥16 and ≥70, respectively. Pubertal stage was assessed using the self-report Pubertal Development Scale [34] (PDS), according to the following categories: 1) prepubertal, 2) early pubertal, 3) midpubertal, 4) late pubertal, and 5) postpubertal. HC-participants were excluded when criteria for a (history of) DSM-IV diagnosis or (sub)clinical scores on clinical questionnaires were met.

Ethics

The EPISCA study was approved by the medical ethics committee of Leiden University Medical Center. All participants provided informed consent according to the Declaration of Helsinki; both participants and parents signed the informed consent form. All anatomical scans were reviewed by a radiologist.

Analysis of demographic data and symptom severity

To examine differences in demographic data and within-subject changes of symptoms in the INT group, (paired) t-tests or two-sided Fisher exact tests were used for continuous and dichotomous data respectively. For non-normally distributed data, as defined by a significant Shapiro-Wilk test, the Mann-Whitney U test or Wilcoxon signed-rank test were used. Incidental missing values on the self-report questionnaires were replaced using expectation maximization (see Online Resource Methods). The Bonferroni method was used to correct p-values for multiple comparisons of the symptom-related questionnaires (4 tests, being comparisons on each timepoint of scores on the RCADS and CDI between the INT and HC group, corrected p-value = 0.0125).

MRI data acquisition

DTI data were collected using a Philips 3.0 T Achieva MRI scanner (Philips Medical Systems, The Netherlands) with an eight-channel sensitivity encoding (SENSE) head coil. A single-shot echo–planar imaging (EPI) sequence was used with the following scan parameters: single band, repetition time = 11 000 ms, echo time = 56 ms, flip angle = 90°, b factor = 1000 s/mm^2, voxel dimensions = 2.3 mm isotropic, number of slices = 73, no slice gap. DTI data were acquired along 32 directions, together with a baseline image without diffusion weighting (b = 0). Total scan time was approximately 7 minutes. In addition, a sagittal 3-dimensional gradient-echo T1-weighted image was acquired for registration purposes with the following scan parameters: TR = 9.8 ms; TE = 4.6 ms; flip angle = 8°; 192 x 152 matrix; FOV = 224 x 177 x 168 mm, 140 sagittal slices; no slice gap; 1.16 x 1.16 x 1.20 mm voxels. Prior

to scanning, all participants were introduced to the scanning situation by lying in a dummy scanner and hearing scanner sounds. All participants were scanned within 2 weeks of initial screening and were new to MRI-scanning procedures.

Processing of DTI data

Image pre-processing and analyses were performed using the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL), version 6.0.3 [35]. The Brain Extraction Tool (BET) was used to remove non-brain tissue from the non-diffusion images. Image distortion and motion artefacts induced by eddy currents, inter-volume and intra-volume head motions were corrected and outliers were replaced with Gaussian Process predictions [36]. Image quality was statistically evaluated afterwards [37]. Individual FA images and primary (l_1), secondary (l_2) and tertiary (l_3) eigenvalues were created by fitting a tensor model to the raw diffusion data using FMRIB's Diffusion Toolbox (FDT).

Standard protocols designed to facilitate harmonized image analysis across multiple sites (http://enigma.ini.usc.edu/protocols/dti-protocols/) were used to visually and statistically evaluate individual vector and raw FA images. These protocols are designed for mega- and meta-analyses within working groups of the ENIGMA consortium (Thompson et al., 2014; Thompson et al., 2020). All DTI data in the present study were collected in one MRI scanner.

Afterwards, a study specific template was created and registered to MNI standard space (see Online Resource Methods). Individual maps of diffusivity measures were calculated using the eigenvalues and aligned onto the template, defining axial diffusivity (AD) as l_1, radial diffusivity (RD) as $l_{23} = (l_2 + l_3) / 2$ and mean diffusivity (MD) as $l_{123} = (l_1 + l_2 + l_3) / 3$. Then, using TBSS, all participants' FA and non-FA images were projected onto the template [38].

Subsequently, quality control was performed twofold: we visually inspected the registered images for misalignment onto the skeleton and calculated individual projection distances of the extracted skeletons onto the template to detect outliers. Outliers were defined as individual projection distance to the template exceeding the threshold of 3.8 mm, which could represent bad alignment to the template [39]. All images were well aligned, and no outliers were detected.

Tracts of interest (TOI)

Based on previous literature, three tracts of interest (TOI) were combined in one binary mask, being the UF (bilateral), cingulum (bilateral) and corpus callosum (genu, body and splenium) using the Johns Hopkins University (JHU) ICBM-DTI-81 white-matter labels atlas [40] provided by FSL. Subsequently, this mask was combined with the mean FA skeleton to include only voxels comprised in both the tract and the skeleton. This confined the statistical analysis to voxels from the center of the tract, thereby minimizing anatomic inter-subject variability, deviations in registration and partial volume effects [41].

Statistical analysis of DTI data

First, we examined voxelwise changes in FA over time between patients and controls by conducting a multi-level block permutation analysis to allow permutation inference with repeated measures data, using 5000 permutations and threshold-free cluster enhancement (TFCE) [42, 43]. Three contrasts were investigated: i) change over time within the INT group (T2 > T1 and T1 > T2), ii) change over time within the HC group (T2 > T1 and T1 > T2), and iii) a group x time interaction using an F-test. A Bonferroni correction of 0.05 / 2 = 0.025 was applied to correct the first two contrasts assessing changes within group over time.

Second, we examined voxelwise associations between longitudinal changes in FA and changes in anxiety and depressive symptoms using FSL's randomise with 5000 permutations and TFCE [43]. Changes in symptom severity were calculated for each patient by subtracting baseline scores on the self-report questionnaires from scores after three months (ΔCDI, ΔRCADS), where a negative Δ-score implied a decrease in self-reported symptoms. Similarly, the change in FA (ΔFA) was calculated by subtracting individual voxelwise values of FA on baseline from voxelwise FA values after three months. Then, associations between ΔFA and i) ΔCDI and ii) ΔRCADS were investigated in separate models. For each analysis, gender, age and puberty stage at baseline (centered) were modelled as covariates of no interest and variance smoothing of sigma = 2.1 mm and family-wise error (FWE) correction of $p < 0.05$ were applied.

Third, we investigated within-subject in the INT group whether baseline FA showed an association with changes in anxious (ΔRCADS) and depressive symptoms (ΔCDI) as described above. Again, for each analysis, gender, age and puberty stage at baseline (centered) were modelled as covariates of no interest and variance smoothing of sigma = 2.1 mm and FWE correction of $p < 0.05$ were applied.

In all analyses, we first investigated FA, as this is a general indicator of WM microstructure. Additional parameters (AD, MD and RD) were examined in significant clusters only. Analyses were conducted within the a-priori defined TOIs and in exploratory whole brain analyses to investigate changes outside the TOI. The JHU ICBM-DTI-81 white-matter labels atlas and JHU white-matter tractography atlas were used to locate significant findings [40]. Participants were included in the respective association analyses if the questionnaire (RCADS or CDI) was filled out on baseline and after three months.

Results

Sample

Two participants (n = 1 INT, n = 1 HC) were excluded due to an anomaly on the structural T1-scan and one HC participant was excluded as criteria for clinical psychopathology were met. Six participants (n = 3 INT, n = 3 HC) were excluded due to technical issues and an additional six participants did not follow up after the first visit (n = 4 INT, n = 2 HC). Thus, the final sample for initial analysis consisted of 47 participants (n = 22 INT, n = 25 HC).

After pre-processing, data from n = 43 participants (n = 22 INT, n = 21 HC) were available for further analysis, as for one HC participant BET was unable to adequately extract non-brain tissue images and an additional three HC participants had to be excluded due to excessive head motion (defined as relative head motion with respect to the previous volume > 2.5 mm).

Demographics

Sample characteristics are summarized in Table 1. Participants in the INT group reported significantly higher levels of internalizing symptoms (self-reported anxiety and depressive symptoms) and puberty stage compared to their healthy peers at both time points. They did not, however, differ with respect to gender distribution, age, IQ or days between visits. Within the INT group, participants reported a significant decrease in depressive symptoms ($T = 50.00$, $z = -2.05$, $p = 0.04$; Wilcoxon signed-rank test) but not in anxiety symptoms after three months ($t(20) = 1.33$, $p = 0.20$; paired t-test).

Table 1 Demographic characteristics of participants with and without clinical depression and comorbid anxiety.

	INT (n = 22)	HC (n = 21)	Statistical analysis
Primary diagnoses[a] (n)			
MDD + GAD	14		
MDD + SAD	14		
MDD + specific phobia	9		
MDD + panic disorder	5		
MDD + separation anxiety	9		
Demographics			
Age in years (mean ± SD; range)	15.93 ± 1.45 (13.23–17.99)	15.09 ± 1.80; (12.33-17.80)	t(41) = 1.68, p = 0.10
Male / Female (n)	2 / 20	4 / 17	p = 0.41, OR = 1.08, 95% CI = 0.24-4.76
IQ (mean ± SD)	105.18 ± 8.17	106.67 ± 8.36	t(36) = 0.59, p = 0.56
Puberty stage (median ± SD)	4.00 ± 0.73	4.00 ± 0.73	U = 253.00, z = 2.32, p = 0.03
Days between visits (median ± SD)	100.00 ± 10.72	98.00 ± 12.48	U = 271.00, z = .98, p = 0.33
Ethnicity (% white)	90.00	96.88	p = 0.61, OR = 3.44, 95% CI = 0.34-35.09
Self-report measures (median ± SD)			
CDI on baseline	20.00 ± 8.91	4.00 ± 3.48	U = 419.50, z = 4.59, p < 0.001***
CDI after three months[b]	11.00 ± 9.12	2.00 ± 3.84	U = 371.00, z = 4.22, p < 0.001***
RCADS on baseline	33.00 ± 15.01	9.00 ± 10.63	U = 374.50, z = 3.90, p < 0.001***
RCADS after three months[c]	25.00 ± 15.32	10.00 ± 8.66	U = 359.50, z = 3.90, p < 0.001***
Secondary (externalizing) diagnoses (n)			
ADHD; inattention	1		
ADHD; hyperactive	1		
Oppositional Defiant Disorder	1		
Behavioral Disorder	2		

MDD: Major Depressive Disorder; GAD: Generalized Anxiety Disorder; SAD: Social Anxiety Disorder; ADHD: Attention Deficit Hyperactivity Disorder; CDI: Children's Depression Inventory; RCADS: Revised Child Anxiety and Depression Scale; OR: unadjusted Odds Ratio; CI: Confidence Interval; SD: Standard Deviation. [a] Diagnoses are not mutually exclusive. [b] Two patients did not complete the questionnaire. [c] One patient did not complete the questionnaire. *** Significant at Bonferroni corrected p-value of 0.0125.

DTI analyses

Significant results are summarized in Table 2 and illustrated in Figure 1 and Online Resource Figures S1 and S2. Permutation analyses did not reveal significant differences in FA over time within the INT or HC group or a significant time x group interaction. In addition, whole brain and TOI analyses did not show significant associations between ΔFA and ΔCDI or between ΔFA and ΔRCADS within the INT group.

Whole brain voxelwise analyses within the INT group revealed that baseline FA in the right posterior corona radiata (PCR; TFCE and FWE corrected p = 0.03) and a small peripheral cluster in the cingulum (TFCE and FWE corrected p = 0.04) were positively associated with ΔCDI (Table 2). Additional permutation analyses in the PCR further revealed that baseline RD (TFCE and FWE corrected p < 0.001) and MD (TFCE and FWE corrected p = 0.01) were significantly negatively associated with ΔCDI. AD was not significantly associated with ΔCDI. In other words: lower baseline FA and higher baseline MD and RD were associated with a higher decrease in self-reported depressive symptoms, as defined by a negative ΔCDI score. Data did not reveal significant associations between ΔCDI and additional WM parameters in the cingulum or significant associations between baseline FA and ΔRCADS.

Interestingly, participants who reported ≥5 points lower on the CDI after three months compared to baseline had significantly lower FA on baseline in the PCR (ΔCDI scores ≤ -5; n = 9, mean ΔCDI ± SD = -10.55 ± 4.91; mean FA ± SD = 0.52 ± 0.06) compared to those who did not change more than ≤ -5 or even had positive ΔCDI scores (n = 11, mean ΔCDI ± SD = 1.64 ± 4.64; mean FA ± SD = 0.57 ± 0.04; t(14) = 2.42, p = 0.03; illustrated in Figure 1B and Online Resource Figure S2).

Table 2 Significant associations between baseline FA and longitudinal changes in depressive symptoms within adolescents with depression and comorbid anxiety.

Cluster index	Voxels (mm^3)	p	Peak MNI coordinates			Location
			x	*y*	*z*	
2	924	0.03	20	-39	39	Right corona radiata (posterior)
1	122	0.04	16	31	32	Right cingulum (cingulate gyrus)

Threshold-free cluster enhancement (TFCE) and family-wise error (FWE) corrected at p-values < 0.05.

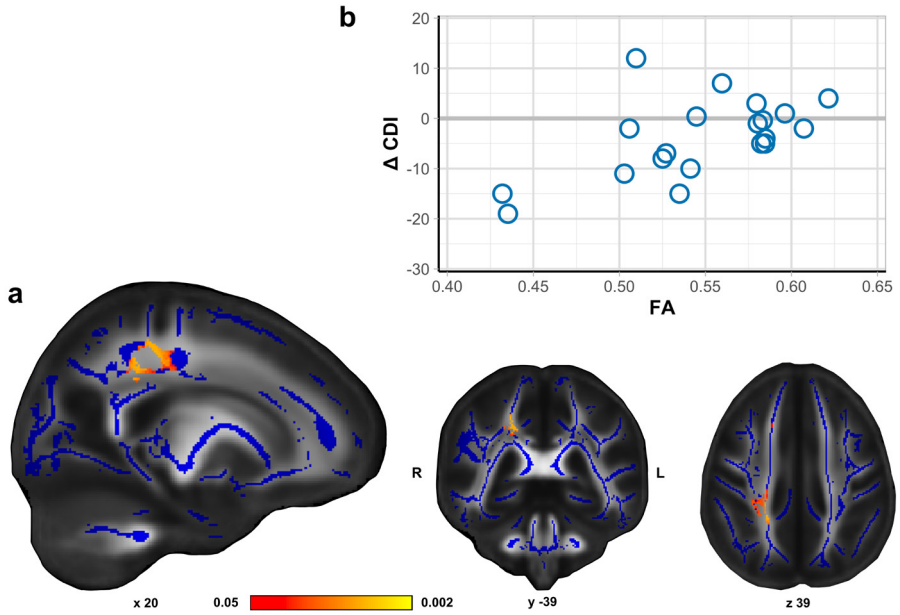

Figure 1 Significant associations between baseline FA and longitudinal changes in depressive symptoms within adolescents with depression and comorbid anxiety. A) Significant cluster from voxelwise whole brain analyses of fractional anisotropy (FA). Sagittal, coronal and axial sections of the WM skeleton (blue), with a subregion of the posterior corona radiata (PCR) showing significant associations of baseline fractional anisotropy (FA) with longitudinal changes in depressive symptoms within adolescents with depression and comorbid anxiety ($p < 0.05$, threshold-free cluster enhancement (TFCE) and family-wise error (FWE) corrected (yellow/orange)). The color bar indicates p-values. B) Association between baseline fractional anisotropy in the PCR and longitudinal changes in depressive symptoms within adolescents with depression and comorbid anxiety. CDI: children's depression inventory; FA: fractional anisotropy.

Discussion

In the present naturalistic study, we investigated the longitudinal course of parameters of WM microstructure in a group of adolescents with clinical depression and anxiety (INT) and compared these to a group of healthy peers (HC), using voxelwise TOI-based and exploratory whole-brain analyses. Furthermore, we examined within-subject associations between clinical symptoms and WM parameters in the INT group: we explored associations between changes in WM parameters and changes in clinical symptoms, and we investigated whether baseline WM parameters were associated with subsequent changes in clinical symptoms over a three-month period. As far as we are aware, this is the first longitudinal DTI study investigating WM microstructure in a naturalistic cohort of adolescents with clinical depression and anxiety, thus providing a first step in exploring WM microstructure in the course of these impairing mental illnesses.

Data revealed no significant changes in FA within and between the INT group and HC group over a three-month period; neither did we find evidence for significant correlations between changes in FA and changes in self-reported internalizing symptoms in the INT group. Voxelwise whole brain analyses

revealed that lower baseline values of FA in the right posterior corona radiata (PCR) were associated with a higher decrease in depressive symptoms after three months. Additional analyses showed that higher baseline values of MD and RD in the PCR were associated with a higher decrease in depressive symptoms. Furthermore, lower baseline values of FA in the right cingulum were associated with a higher decrease in depressive symptoms after three months, but no associations between depressive symptoms and other WM parameters in this cluster were found.

The corona radiata is part of the limbic-thalamo-cortical circuitry and has been implicated in several psychiatric disorders such as major depressive disorder and schizophrenia [44, 45]. It consists of several white matter tracts, such as the superior corona radiata (SCR) and posterior corona radiata (PCR). Lower FA in the SCR has been associated with an increased risk of psychopathology in a sample of healthy adolescents with a familial history of internalizing and/or externalizing psychopathology [46]. The PCR consists of ascending fibers connecting the thalamus with the cerebral cortex, and descending fibers connecting the frontoparietal cortex with subcortical nuclei and the spinal cord, including areas functionally involved in the default mode network, which regulates attention during cognitive performance, and in top-down control of emotional experience and mood [47, 48]. In general, low FA coupled with high MD and RD and unchanged AD could be indicative of demyelination, low axonal packaging or less spatial coherency of fiber alignment [21, 49]. Thus, our results might point at less spatial coherency in the PCR, as bundles of the corona radiata follow a geometrically complex path with a high degree of intra-voxel changes in fiber orientation before reaching the corpus callosum [48, 50]. However, the role and function of the PCR in the onset and development of adolescent depression and anxiety is yet to be further unraveled.

The cingulum, the other track implicated in this study, is functionally involved in among others regulation of emotional states. Anatomically, the cingulum bundle runs in the cingulate gyrus from anterior to posterior sections of the brain and is thought to mediate communication between the cingulate gyrus and regions involved in the limbic system, as well as within regions of the cingulate gyrus [12, 47]. Previous research has implicated lower FA in the cingulum in adolescent depression compared to healthy controls [10, 27]. In addition, decelerated maturation of myelin and lower age-related FA increase in the cingulum has been associated with anxiety symptoms in healthy adolescents [51, 52]. These results could imply a role of the cingulum in impaired regulation of emotional states, contributing to onset and persistence of internalizing disorders. However, as we discovered associations between lower baseline FA and changes in depressive symptoms without associations with other WM parameters in a small cluster (size: 122 voxels), directionality of underlying WM microstructure and thus interpretation of this finding is limited [21, 49].

Data did not reveal any significant associations between changes in FA and changes in self-reported symptoms. Since WM development is associated with learning new skills, this finding was unexpected. For example, studies in mice reported on FA increases after learning a new skill and steeper FA development curves were associated with higher performance [53, 54]. In agreement with this, knock-out mice showed

impairment in acquiring new skills when no myelination was possible [55]. We hypothesize that three months was too short to detect any significant changes; alternatively, our sample might have been too small to achieve sufficient power to detect significant associations due to type 2 errors.

There are some more limitations to consider. First, only a few boys participated in this study although the gender distribution was not significantly different between the clinical and healthy control group. However, as girls are more likely to develop internalizing psychopathology, and the female gender has been associated with an increased risk on comorbid anxiety and depression, we feel our study is representative of the clinical population [1]. Second, sample size and study duration were modest. Yet, previous longitudinal studies in adolescents with internalizing disorders have assessed longitudinal effects of cognitive behavioral therapy (CBT) over three months and reported functional brain changes in amygdalar – prefrontal connections, which in some studies were associated with improvement of clinical symptoms [15, 56-58]. In addition, the present design does not allow to differentiate between symptoms of clinical anxiety and depression. Furthermore, several studies concerning white matter development in healthy adolescents have reported continuing white matter microstructure developmental changes during adolescence well into young adulthood [59-61]. Taken together, we expected three months to be sufficient to detect any changes. Our modest sample size could have induced type 2 errors. Indeed, perhaps our study had too little power to detect a significant effect. Furthermore, treatment was not standardized, but all participants were treatment-naïve at baseline, thus enabling us to examine the course of symptoms in this clinically representative population. Lastly, we did not account for social-economic status or illness duration.

To better understand the course and neurodevelopment of WM in adolescent anxiety and depression, we recommend future studies to use a longitudinal study design with larger groups of patients and healthy controls and a standardized treatment in an elongated study period, as standardization of treatment would aid the interpretation of intervention effects on the course of underlying microstructure of WM tracts.

In conclusion, this study revealed no significant changes in WM characteristics within and between adolescents with internalizing disorders and healthy peers over a three-month period; neither were significant correlations between changes in FA and changes in self-reported internalizing symptoms in adolescents with internalizing disorders found. However, analysis revealed associations between parameters of WM microstructure and changes in self-reported depressive symptoms in adolescents with clinical depression and comorbid anxiety. We found an association between a higher decrease in depressive symptoms over a period of three months, and characteristics of baseline WM microstructure in the posterior corona radiata (lower FA, higher RD and MD). These results further entangle the contribution of underlying neurobiological factors in the course of adolescent depression and comorbid anxiety disorders.

Supplemental methods

Statistical analysis: expectation maximization

A limited number of items were missing in the CDI questionnaire (10 items in total, 6 items for 6 participants at session 1 and 4 items for 1 participant at session 2) and in the RCADS (4 items in total, 3 items for 3 participants at session 1 and 1 item for 1 participant at session 2). Expectation maximization was allowed as Little's MCAR tests showed that data was completely missing at random for both questionnaires at both sessions.

DTI registration

DTI-TK uses tensor-based registration and has proven additional value to TBSS [62, 63]. In the present study, a within-subject template was created for each subject by computing an initial average template from the two time points. The template was iteratively refined using the following procedure: the within-subject tensor images were registered to the template and a refined template was computed as an average of the registered tensor images for the next iteration. The process was repeated until the change between templates from consecutive iterations became sufficiently small, first with affine and then with non-linear registrations. Once a suitable non-linear alignment has been established, a study-specific (group-wise) template was created from all of the within-subject templates using the described iterative method. Afterwards, this improved study-specific template was resampled to isotropic $1mm^3$ resolution and each original within-subject tensor image was registered to the study-specific template using non-linear alignments. Finally, the FA maps and refined scalar diffusivity maps (e.g. the eigenvalues and eigenvectors) were obtained for each participant using singular-value decomposition. To warp all DTI images to standard space, the FA map of the final study-specific tensor template was linearly registered to the ENIGMA DTI-MNI-space [64].

Supplemental results

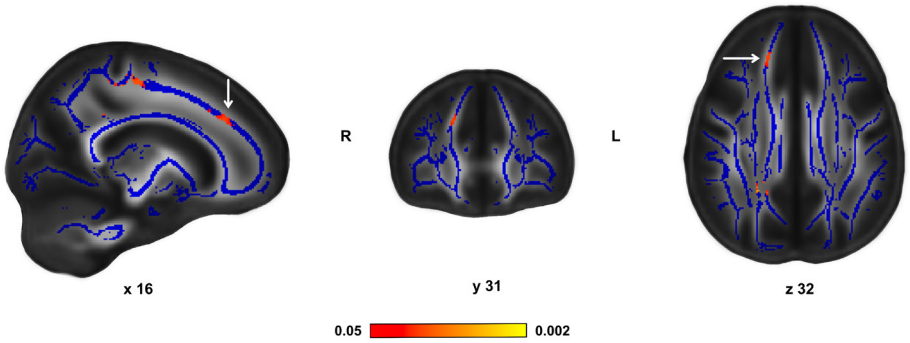

Figure S1 Significant associations between baseline FA in the cingulum and longitudinal changes in depressive symptoms within adolescents with depression and comorbid anxiety. Significant cluster from voxelwise whole brain analyses of fractional anisotropy (FA). Sagittal, coronal and axial sections of the WM skeleton (blue), with a subregion of the cingulum (indicated by white arrow) showing significant associations of fractional anisotropy (FA) with longitudinal changes in depressive symptoms within adolescents with depression and comorbid anxiety ($p < 0.05$, threshold-free cluster enhancement (TFCE) and family-wise error (FWE) corrected (yellow/orange)). The color bar indicates p-values. The cluster not indicated by arrow depicts other the significant cluster in the posterior corona radiata shown in Figure 1 in main article.

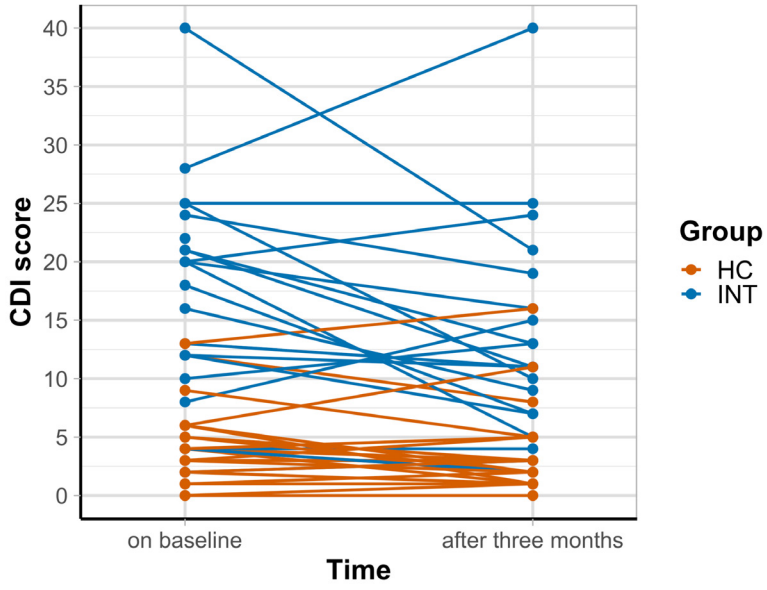

Figure S2 Association between baseline fractional anisotropy in the PCR and longitudinal changes in depressive symptoms within adolescents with depression and comorbid anxiety. CDI: children's depression inventory; FA: fractional anisotropy.

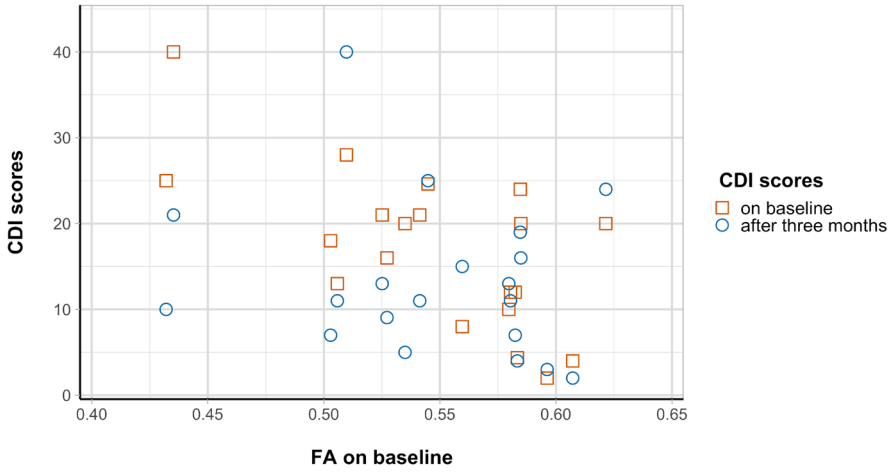

Figure S3 Longitudinal changes in depressive symptoms within adolescents with depression and comorbid anxiety and healthy control participants. CDI: children's depression inventory; HC: healthy control participants; INT: adolescents with depression and comorbid anxiety.

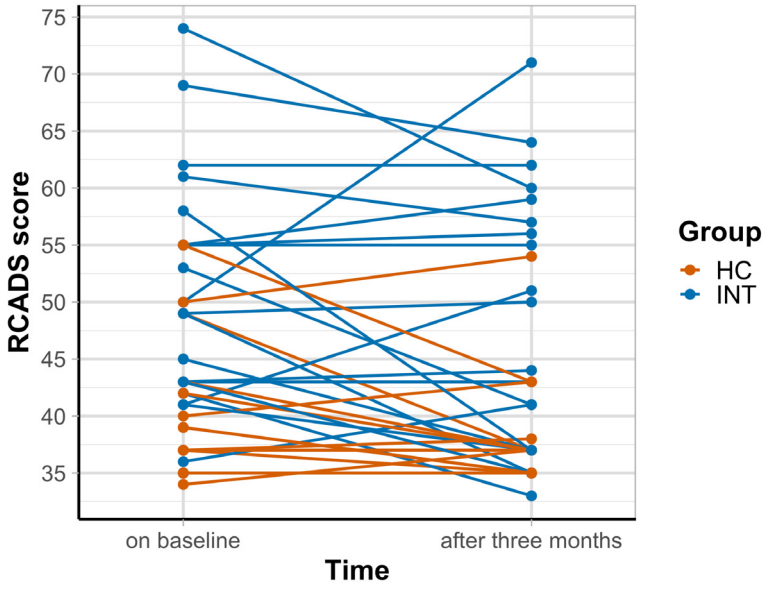

Figure S4 Longitudinal changes in anxiety symptoms within adolescents with depression and comorbid anxiety and healthy control participants. RCADS: revised child anxiety and depression scale; HC: healthy control participants; INT: adolescents with depression and comorbid anxiety.

Table S1a Overview of literature; demographic information.

Study	Author	Contrast	Patients	Age (years)	Anxiety symptoms	Depressive symptoms	Illness (months)	HC	Age (years)	Anxiety symptoms	Depressive symptoms
1	Bessette et al. [7]	MDD vs. HC	7M, 24F	17.1±1.9	n.a.	BDI-II: 26.3±11.2	n.a.	12M, 19F	17.0±2.4	n.a.	BDI: 3.1±3.0
2	Aghajani et al. [9]	MDD vs. HC	4M, 21F	15.6±1.4	RCADS anx: 31.5±5.7	CDI: 18.6±9.7	n.a.	3M, 18F	14.7±1.6	RCADS anx: 14.4±9.7	CDI: 4.6±3.3
3	LeWinn et al. [10]	MDD vs. HC	21M, 31F	16.2±0.2	RCADS tot: 65.8±1.0	CDRS: 72.5±11	n.a.	16M, 26F	16.0±0.2	RCADS tot: 41.7±1.2	CDRS: 33.0±0.7
4	Liao et al. [11]	GAD vs. HC	13M, 11F	17.0±0.7	PSWQ: 54.6±8.9	n.a.	n.a.	13M, 11F	16.6±0.8	SCARED: < 25	n.a.
5	Cullen et al. [8]	MDD vs. HC	4M, 10F	16.8±1.2	n.a.	BDI-II: 29.0±11.8	33.9±20.2	6M, 8F	16.8±1.5	n.a.	n.a.

Results are reported in mean ± SD. HC: healthy control. MDD: major depressive disorder. GAD: generalized anxiety disorder. F: female. M: male. BDI: Beck's Depression Inventory. RCADS: Revised Child Anxiety and Depression Scale. CDRS: Children's Depression Rating Scale. SCARED: Child Anxiety Related Emotional Disorders. PSWQ: The Penn State Worry Questionnaire.

Table S1b Overview of literature; findings.

Study	Variables	CC			PLIC		EC		ALIC		CR		ACR		PCR		SCR		SLF		ILF		IFOF		UF		ATR		PTR		PC		MFPC	
		G	B	S	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R
1	FA TBSS, MDD vs HC	↓	↓	ø	↓	↓	↓	ø	↓	↓	ø	ø	↓	↓	ø	ø	↓	↓	ø	ø	↓	ø	↓	↓	↓	ø	ø	↓	↓	ø	↓	ø	↓	↓
2	FA TBSS, MDD vs HC	ø	↓	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø
	FA TOI, MDD vs HC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑	-	-	-	-	-	-	-	-	-
	FA TBSS, MDD vs HC	↓	↓	ø	ø	ø	↓	↓	↓	↓	ø	ø	↓	↓	ø	ø	↓	↓	ø	ø	ø	ø	↓	↓	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø
3	Tractography, MDD vs HC	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	↓	↓	ø	ø	ø	ø	ø	ø	ø	ø
	FA TBSS, GAD vs HC	ø	ø	ø	ø	↓	ø	ø	↓	ø	↓	↓	ø	ø	ø	ø	ø	ø	↓	ø	↓	↓	↓	↓	↓	↓	ø	ø	ø	ø	ø	ø	ø	ø
4	FA TBSS, ass w/ PSWQ	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø
5	FA TBSS, MDD vs HC (uncorr)	↓	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	↓	ø	ø	ø	↓	↓	↓	↓	ø	ø	ø	ø	ø	ø	ø	ø

CC: corpus callosum; G: genu; B: body; S: splenium; PLIC: posterior limb of internal capsula; EC: external capsula; ALIC: anterior limb of internal capsula; CR: corona radiata; ACR: anterior corona radiata; PCR: posterior corona radiata; SCR: superior corona radiata; SLF: superior longitudinal fasciculus; ILF: inferior longitudinal fasciculus; IFOF: inferior fronto-occipital fasciculus; UF: uncinate fasciculus; ATR: anterior thalamic radiation; PTR: posterior thalamic radiation; PC: posterior cingulum; MFPC: medial frontal pregenual cingulate.

Table S2 Overview of treatments received by participants.

Combination of treatments	Number of participants (n)
CBT individual	5
CBT group	1
CBT individual + systemic family therapy	7
CBT individual + systemic therapy, parents only	4
CBT individual+ EMDR	1
CBT individual + CBT group + systemic family therapy	1
CBT group + systemic family therapy	1
Creative therapy + systemic therapy, parents only	1
None started	1

CBT: cognitive behavioral therapy. EMDR: Eye Movement Desensitization and Reprocessing. Creative therapy was received in a group setting.

References

1. Costello, E.J., et al., *Prevalence and development of psychiatric disorders in childhood and adolescence.* Arch Gen Psychiatry, 2003. **60**(8): p. 837-44.
2. Melton, T.H., et al., *Comorbid Anxiety and Depressive Symptoms in Children and Adolescents: A Systematic Review and Analysis.* J Psychiatr Pract, 2016. **22**(2): p. 84-98.
3. Swartz, J.R. and C.S. Monk, *The role of corticolimbic circuitry in the development of anxiety disorders in children and adolescents.* Curr Top Behav Neurosci, 2014. **16**: p. 133-48.
4. Toenders, Y.J., et al., *Neuroimaging predictors of onset and course of depression in childhood and adolescence: A systematic review of longitudinal studies.* Dev Cogn Neurosci, 2019. **39**: p. 100700.
5. Strawn, J.R., et al., *Neurobiology of Pediatric Anxiety Disorders.* Curr Behav Neurosci Rep, 2014. **1**(3): p. 154-160.
6. Tseng, W.L., E. Leibenluft, and M.A. Brotman, *A systems neuroscience approach to the pathophysiology of pediatric mood and anxiety disorders.* Curr Top Behav Neurosci, 2014. **16**: p. 297-317.
7. Bessette, K.L., et al., *White matter abnormalities in adolescents with major depressive disorder.* Brain Imaging Behav, 2014. **8**(4): p. 531-41.
8. Cullen, K.R., et al., *Altered white matter microstructure in adolescents with major depression: a preliminary study.* J Am Acad Child Adolesc Psychiatry, 2010. **49**(2): p. 173-83 e1.
9. Aghajani, M., et al., *Altered white-matter architecture in treatment-naive adolescents with clinical depression.* Psychol Med, 2014. **44**(11): p. 2287-98.
10. LeWinn, K.Z., et al., *White matter correlates of adolescent depression: structural evidence for frontolimbic disconnectivity.* J Am Acad Child Adolesc Psychiatry, 2014. **53**(8): p. 899-909, 909 e1-7.
11. Liao, M., et al., *White matter abnormalities in adolescents with generalized anxiety disorder: a diffusion tensor imaging study.* BMC Psychiatry, 2014. **14**: p. 41.
12. Schmahmann, J.D., et al., *Association fibre pathways of the brain: parallel observations from diffusion spectrum imaging and autoradiography.* Brain, 2007. **130**(Pt 3): p. 630-53.
13. Schmahmann, J.D. and D.N. Pandya, *Fiber Pathways of the Brain, in Fiber Pathways of the Brain.* 2006. p. 409-414.
14. Chattopadhyay, S., et al., *Cognitive Behavioral Therapy Lowers Elevated Functional Connectivity in Depressed Adolescents.* EBioMedicine, 2017. **17**: p. 216-222.
15. Connolly, C.G., et al., *Resting-state functional connectivity of the amygdala and longitudinal changes in depression severity in adolescent depression.* J Affect Disord, 2017. **207**: p. 86-94.
16. Cullen, K.R., et al., *Neural Correlates of Antidepressant Treatment Response in Adolescents with Major Depressive Disorder.* J Child Adolesc Psychopharmacol, 2016. **26**(8): p. 705-712.
17. Pannekoek, J.N., et al., *Aberrant resting-state functional connectivity in limbic and salience networks in treatment-naive clinically depressed adolescents.* J Child Psychol Psychiatry, 2014. **55**(12): p. 1317-27.
18. Pannekoek, J.N., et al., *Reduced anterior cingulate gray matter volume in treatment-naive clinically depressed adolescents.* Neuroimage Clin, 2014. **4**: p. 336-42.
19. van den Bulk, B.G., et al., *Amygdala activation during emotional face processing in adolescents with affective disorders: the role of underlying depression and anxiety symptoms.* Front Hum Neurosci, 2014. **8**: p. 393.
20. van den Bulk, B.G., et al., *Amygdala habituation to emotional faces in adolescents with internalizing disorders, adolescents with childhood sexual abuse related PTSD and healthy adolescents.* Dev Cogn Neurosci, 2016. **21**: p. 15-25.
21. Alexander, A.L., et al., *Diffusion tensor imaging of the brain.* Neurotherapeutics, 2007. **4**(3): p. 316-29.
22. Hasan, K.M., A.L. Alexander, and P.A. Narayana, *Does fractional anisotropy have better noise immunity characteristics than relative anisotropy in diffusion tensor MRI? An analytical approach.* Magn Reson Med, 2004. **51**(2): p. 413-7.
23. Budde, M.D., et al., *Axial diffusivity is the primary correlate of axonal injury in the experimental autoimmune encephalomyelitis spinal cord: a quantitative pixelwise analysis.* J Neurosci, 2009. **29**(9): p. 2805-13.
24. Song, S.K., et al., *Demyelination increases radial diffusivity in corpus callosum of mouse brain.* Neuroimage, 2005. **26**(1): p. 132-40.
25. Horsfield, M.A. and D.K. Jones, *Applications of diffusion-weighted and diffusion tensor MRI to white matter diseases - a review.* NMR Biomed, 2002. **15**(7-8): p. 570-7.
26. Kochunov, P., et al., *Relationship between white matter fractional anisotropy and other indices of cerebral health in normal aging: tract-based spatial statistics study of aging.* Neuroimage, 2007. **35**(2): p. 478-87.
27. Bessette, K.L., et al., *White matter abnormalities in adolescents with major depressive disorder.* Brain Imaging Behav, 2014. **8**(4): p. 531-41.
28. van den Bulk, B.G., et al., *How stable is activation in the amygdala and prefrontal cortex in adolescence? A study of emotional face processing across three measurements.* Dev Cogn Neurosci, 2013. **4**: p. 65-76.
29. Wechsler, D., *Manual for the Wechsler Intelligence Scale for Children - Third Edition (WISC-III).* 1991, San Antonio, TX: The Psychological Corporation.
30. Wechsler, D., *Wechsler Adult Intelligence Scale.* 3rd ed. 1997,

San Antonio, TX: Harcourt Assessment.

31. Silverman, W. and A.M. Albano, *The anxiety disorders interview schedule for DSM-IV - Child and parent versions*. 1996, San Antonio, TX: Raywind Publications.
32. Kovacs, M., *The Children's Depression, Inventory (CDI).* Psychopharmacol Bull, 1985. **21**(4): p. 995-8.
33. Chorpita, B.F., et al., *Assessment of symptoms of DSM-IV anxiety and depression in children: a revised child anxiety and depression scale.* Behav Res Ther, 2000. **38**(8): p. 835-55.
34. Petersen, A.C., et al., *A self-report measure of pubertal status: Reliability, validity, and initial norms.* J Youth Adolesc, 1988. **17**(2): p. 117-33.
35. Smith, S.M., et al., *Advances in functional and structural MR image analysis and implementation as FSL.* Neuroimage, 2004. **23 Suppl 1**: p. S208-19.
36. Andersson, J.L.R. and S.N. Sotiropoulos, *An integrated approach to correction for off-resonance effects and subject movement in diffusion MR imaging.* Neuroimage, 2016. **125**: p. 1063-1078.
37. Bastiani, M., et al., *Automated quality control for within and between studies diffusion MRI data using a non-parametric framework for movement and distortion correction.* Neuroimage, 2019. **184**: p. 801-812.
38. Smith, S.M., et al., *Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data.* Neuroimage, 2006. **31**(4): p. 1487-505.
39. Acheson, A., et al., *Reproducibility of tract-based white matter microstructural measures using the ENIGMA-DTI protocol.* Brain Behav, 2017. **7**(2): p. e00615.
40. Mori, S., et al., *MRI Atlas of Human White Matter.* 2005, Amsterdam: Elsevier.
41. Westlye, L.T., et al., *Error-related negativity is mediated by fractional anisotropy in the posterior cingulate gyrus--a study combining diffusion tensor imaging and electrophysiology in healthy adults.* Cereb Cortex, 2009. **19**(2): p. 293-304.
42. Winkler, A.M., et al., *Multi-level block permutation.* Neuroimage, 2015. **123**: p. 253-68.
43. Winkler, A.M., et al., *Permutation inference for the general linear model.* Neuroimage, 2014. **92**(100): p. 381-97.
44. van Velzen, L.S., et al., *White matter disturbances in major depressive disorder: a coordinated analysis across 20 international cohorts in the ENIGMA MDD working group.* Mol Psychiatry, 2020. **25**(7): p. 1511-1525.
45. Koshiyama, D., et al., *White matter microstructural alterations across four major psychiatric disorders: mega-analysis study in 2937 individuals.* Mol Psychiatry, 2020. **25**(4): p. 883-895.
46. Jones, S.A., A.M. Morales, and B.J. Nagel, *Resilience to Risk for Psychopathology: The Role of White Matter Microstructural Development in Adolescence.* Biol Psychiatry Cogn Neurosci Neuroimaging, 2019. **4**(2): p. 180-189.
47. Catani, M., et al., *Virtual in vivo interactive dissection of white matter fasciculi in the human brain.* Neuroimage, 2002. **17**(1): p. 77-94.
48. Wakana, S., et al., *Fiber tract-based atlas of human white matter anatomy.* Radiology, 2004. **230**(1): p. 77-87.
49. Jones, D.K., T.R. Knosche, and R. Turner, *White matter integrity, fiber count, and other fallacies: the do's and don'ts of diffusion MRI.* Neuroimage, 2013. **73**: p. 239-54.
50. Hasan, K.M., et al., *Diffusion tensor tractography quantification of the human corpus callosum fiber pathways across the lifespan.* Brain Research, 2009. **1249**: p. 91-100.
51. Vanes, L.D., et al., *White matter tract myelin maturation and its association with general psychopathology in adolescence and early adulthood.* Hum Brain Mapp, 2020. **41**(3): p. 827-839.
52. Albaugh, M.D., et al., *Anxious/depressed symptoms are related to microstructural maturation of white matter in typically developing youths.* Dev Psychopathol, 2017. **29**(3): p. 751-758.
53. Sampaio-Baptista, C., et al., *Motor skill learning induces changes in white matter microstructure and myelination.* J Neurosci, 2013. **33**(50): p. 19499-503.
54. Sampaio-Baptista, C., et al., *White matter structure and myelin-related gene expression alterations with experience in adult rats.* Prog Neurobiol, 2020. **187**: p. 101770.
55. Liu, J., et al., *White Matter Plasticity in Anxiety: Disruption of Neural Network Synchronization During Threat-Safety Discrimination.* Front Cell Neurosci, 2020. **14**: p. 587053.
56. Cyr, M., et al., *Altered network connectivity predicts response to cognitive-behavioral therapy in pediatric obsessive-compulsive disorder.* Neuropsychopharmacology, 2020. **45**(7): p. 1232-1240.
57. La Buissonniere-Ariza, V., et al., *Neural correlates of cognitive behavioral therapy response in youth with negative valence disorders: A systematic review of the literature.* J Affect Disord, 2021. **282**: p. 1288-1307.
58. Villa, L.M., et al., *Cognitive behavioral therapy may have a rehabilitative, not normalizing, effect on functional connectivity in adolescent depression.* J Affect Disord, 2020. **268**: p. 1-11.
59. Asato, M.R., et al., *White matter development in adolescence: a DTI study.* Cereb Cortex, 2010. **20**(9): p. 2122-31.
60. Bava, S., et al., *Longitudinal characterization of white matter maturation during adolescence.* Brain Res, 2010. **1327**: p. 38-46.
61. Lebel, C., S. Treit, and C. Beaulieu, *A review of diffusion MRI of typical white matter development from early childhood to young adulthood.* NMR Biomed, 2019. **32**(4): p. e3778.
62. Bach, M., et al., *Methodological considerations on tract-based*

spatial statistics (TBSS). Neuroimage, 2014. **100**: p. 358-69.

63. Zhang, H., et al., *Deformable registration of diffusion tensor MR images with explicit orientation optimization.* Med Image Anal, 2006. **10**(5): p. 764-85.
64. Jahanshad, N., et al., *Multi-site genetic analysis of diffusion images and voxelwise heritability analysis: a pilot project of the ENIGMA-DTI working group.* Neuroimage, 2013. **81**: p. 455-469.