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Uncinate Fasciculus Microstructure in Childhood Anxiety Disorders is Altered in Boys but not Girls

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Abstract

Objective: Anxiety disorders (ADs) are common, can result in life-long suffering, and frequently begin prior to adolescence. Evidence from adults suggests that altered prefrontal-limbic connectivity is a pathophysiological feature of ADs. More specifically, in adults with ADs decreased fractional anisotropy (FA), a measure of white matter integrity, has been observed in the uncinate fasciculus (UF), the major tract that connects limbic and prefrontal regions. Due to the early onset of ADs and the increased incidence in ADs in females during their reproductive years,

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it is important to understand whether the reduction in UF FA exists in children with ADs and the extent to which this alteration is sex related. To address these issues, we assessed FA in the UF, in unmedicated boys and girls with ADs.

Method: FA measures were derived from diffusion tensor images that were acquired from 98 unmedicated children (age 8–12); 52 met criteria for generalized anxiety disorder, separation anxiety disorder, social anxiety disorder or anxiety disorder not otherwise specified, and 46 were matched controls.

Results: Tract-based results demonstrated that AD children have significant reductions in UF FA. A significant sex-by-group interaction and post-hoc testing revealed that this effect was only evident in boys. No other main effects or sex-by-group interactions were found for other white matter tracts.

Conclusions: These findings provide evidence of UF white matter alterations in boys with ADs. The data demonstrate that AD-related alterations in prefrontal-limbic structural connectivity are present early in life, are not related to psychotropic medication exposure, and are sex specific. Building on these findings, future research has the potential to provide insights into the genesis and sexual dimorphism of the pathophysiology that leads to ADs, as well as the identification of sex specific, early life treatment targets.

Introduction

Anxiety disorders (ADs) are among the most common psychiatric disorders and can become chronic[1,2]. ADs affect more than 1 -in-4 individuals at some point in their life and are generally more prevalent in females than in males[3,4]. Individuals suffering from ADs have impairments in psychosocial functioning and quality of life[5], and when severe, ADs can be disabling. Children are especially affected by pathological anxiety, as ADs are among the earliest psychiatric illnesses to develop[2], affecting up to 20% of youth[6–8]. Additionally, anxiety symptoms in children are often comorbid with other conditions, and are strong predictors of the subsequent development of other anxiety disorders, affective disorders, and comorbid substance abuse[2]. While early and effective treatments have the potential to prevent the life long suffering and psychosocial dysfunction associated with these disorders, current treatments for childhood ADs, such as cognitive behavioral therapy and selective serotonin reuptake inhibitors, are suboptimal. Many children fail to respond to these treatments and those that do respond have relatively high rates of relapse[9]. Establishing a better understanding of the pathophysiology of childhood ADs will facilitate the development of novel, more effective treatments[10].

Despite the prevalence and importance of childhood ADs, few studies have focused on characterizing the neural alterations that underlie the early manifestations of these disorders[11]. Current knowledge of the pathophysiology of ADs is mostly derived from studies of adults, with a few studies examining adolescent and preadolescent AD patients[11]. Previous work revealed that ADs are associated with hyperactivation of the amygdala and insula in response to negative emotional stimuli[12–14]. While recent work from our group has shown that amygdala activation is significantly higher in preadolescent children with ADs when confronted with uncertain conditions[15]. We, as well as others, also report decreased

anxiety-related functional coupling between the amygdala and regulatory prefrontal cortical (PFC) regions such as anterior cingulate cortex, orbital frontal cortex (OFC), dorsolateral prefrontal cortex and medial prefrontal cortex[16–19]. Decreased anxiety-related functional coupling between the amygdala and PFC is evolutionarily conserved, as we demonstrated similar findings in a rhesus monkey model of early life anxiety[18].

Complementing these findings, diffusion tensor imaging (DTI) studies in patients with ADs report altered structural connectivity between temporal lobe and PFC regions, revealing that fractional anisotropy (FA) in the uncinate fasciculus (UF) is reduced in AD patients[20–25]. These studies focused predominantly on AD adults, with only 1 study that included adolescent AD patients[25]. The UF is highly relevant to anxiety and emotion regulation as it connects structures that are crucial to affective processing such as the amygdala, entorhinal/perirhinal cortices, and parahippocampal gyrus to frontal regions including the anterior PFC, OFC, ventromedial PFC, anterior cingulate cortex and insula[26,27].

While these structural alterations may reflect the pathophysiology associated with ADs, it is also possible that these alterations result from illness chronicity, medication exposure, and/or other non-pathophysiological factors. Studies in medication-naive children have the advantage of examining white matter pathways early in the illness and in the absence of many of the influences that may indirectly affect white matter. Additionally, these studies may inform mechanisms underlying the childhood risk to develop ADs and may also point to new early life treatment and prevention strategies.

Here, we use DTI to assess white matter integrity in unmedicated preadolescent children with ADs compared to healthy controls, to test the hypothesis that childhood ADs are associated with alterations in the UF FA. Because of known sex differences in the prevalence of ADs[3,4], and the interest in sexual dimorphism in the relation to brain development[28,29], we also examine sex differences in this effect. Additional analyses aimed to further characterize these microstructural alterations across the brain by investigating three supplementary diffusivity measures, as well as investigate interactions with symptoms measures and steroid hormones. As such tract-based analyses of six additional structures for which the relations to ADs have been less consistent[20–25,30,31], as well as whole brain voxel-based analyses were performed.

Methods

Participants

For a detailed description see eMethods. Briefly, diffusion-weighted magnetic resonance imaging (MRI) scans were obtained from a preadolescent sample across two research sites; University of Wisconsin (UW) and National Institute of Mental Health (NIMH). The final sample consisted of 98 children (age 8−12; 46 controls, 50 girls; Table 1) across the two sites. Control subjects were age and sex matched at the group level to the AD sample with no current or past psychiatric illness. Neither the AD patients nor controls were currently on any psychotropic medication, and neither reported any psychotropic medication usage at any point during their life. Informed assent and consent was obtained from all participants and

their parents, in accordance with the institutional review board of UW and NIMH. Individuals were compensated for their time and effort.

All participants underwent a Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime version (K-SADS-PL)[32] that was administered by a trained clinical psychologist (PhD) or psychiatrist (MD), see eMethods for details on cross-rater agreement. Beyond diagnosis, children's symptoms were rated by both the child and a parent using multiple symptom questionnaires (see eMethods).

Data Acquisition & Analyses

DTI acquisition: For detailed description see eMethods. Briefly, brain images at both sites were collected on MRI scanners of the same make and model, with comparable parameters, optimized for diffusion-weighted imaging with 48 directions.

Steroid hormone collection: See eMethods.

DTI analyses: For detailed description see eMethods. Briefly, images were corrected for field inhomogeneity and eddy currents, after which the tensors were calculated using a robust tensor estimation. Tensor images of all subjects were coregistered iteratively using non-linear tensor-based normalization tools and then registered to MNI 152-space. In this template space diffusion measures were extracted to quantify local white matter microstructure. Deterministic fiber tractography was performed to delineate tracts of interest, that besides the UF[20–25] included cortico-limbic association pathways previously implicated in ADs, including the cingulum bundle (CING)[20,30], superior longitudinal fasciculus (SLF)[20,24,25], fornix (FX)[20], and inferior frontal occipital fasciculus (IFO)[20,25]. Tracts shown to have anxiety-related changes in other publications were also extracted, including thalamocortical projection fibers in the internal capsule (IC)[20,25], and interhemispheric commissural fibers of the corpus callosum (CC)[31].

Next, in order to quantify the microstructure of an entire white matter structure a weighted mean was calculated per tract for each diffusion measure per subject. Tract-based analyses were performed with average tract measures predicting AD patient status. To characterize microstructural changes in these white matter pathways beyond FA, analyses assessed mean diffusivity (MD), axial diffusivity (XD) and radial diffusivity (RD) as well. We also examined the possibility of sexually dimorphic effects relating white matter microstructure to anxiety in AD children. To that effect, the interaction between patient status (AD, control) and sex (boys, girls) was tested in the same model. Age, sex and site were included in this model as covariates. Since we had no a *priori* hypothesis about left versus right tract differences, the bilateral components of each tract were combined into one average.

Analyses were run using standard linear regression techniques in R-studio (Version 1.0.136)[33]. To control for familywise error a Sidak correction for the number of tracts was applied where m = 7, for each white matter tract tested, resulting in a corrected two-tailed significance threshold of $\alpha < 0.0073$ ($\alpha_{SID} = 1 - (1 - a)^{1/m} = 1 - (1–0.05)^{1/7} = 0.0073$). The effect sizes of significant tract-based effects were reported using Cohen's d[34–36], to aid in interpretation. Cohen's d values greater than 0.8 are considered a large effect size, while 0.5

is a medium effect[36]. Voxel-based analyses of FA across the brain were performed to test regions within and beyond the a *priori* determined tracts.

Additional analyses, described in detail in the results, included age, sex and site as covariates where applicable, and used standard linear regression techniques in R-studio (Version 1.0.136)[33] and Python.

Steroid hormone analyses: See eMethods.

Results

Demographics, Symptoms & Hormonal Measurements

Ninety-eight children (age 8–12; 50 girls, 46 controls) were included in the final analyses. AD and control children did not significantly differ in age ($t(94) = 0.385$, $p = 0.701$), sex ($t(94) = 0.184$, $p = 0.854$) or IQ ($t(91) = 0.888$, $p = 0.377$; Table 1). Furthermore, there was no significant group difference in physical development as assessed by the Tanner stages ($t(86) = -1.499$, $p = 0.140$; Table 1). Age and sex each significantly predicted Tanner scores (age; $t(86) = 8.510$, $p < 0.001$, sex; $t(86) = -2.006$, $p = 0.048$), with older children and females having significantly higher Tanner scores, but the group by sex interaction was non-significant ($p = 0.406$).

AD subjects had significantly higher symptom scores on all clinical scales (anxiety, depression and ADHD) compared to controls ($ps < 0.001$; Table 1). No group by sex interactions were found for depression and ADHD symptoms. A group by sex interaction approached significance for self-reported anxiety ($t(89) = 1.82$, $p = 0.072$; eFigure 1). *Post hoc* tests indicated that self-reported anxiety scores were significantly lower for AD boys compared to AD girls ($t(48) = 2.49$, $p = 0.016$).

Testosterone was significantly higher in girls compared to boys ($z(55) = -2.57$, $p = 0.006$; for mean values by group for each sex see Table 1), an effect that has previously been observed in pre-adolescent children[37]. Neither estradiol nor cortisol differed by sex ($ps > 0.4$). Age was positively related to testosterone ($z(55) = 3.286$, $p = 0.006$), and estradiol ($z(55) = 2.538$, $p = 0.011$).

While there were no group differences in cortisol or testosterone ($p > 0.22$), an unpredicted significant group effect was observed for estradiol levels, in which AD children had lower levels of estradiol compared to controls ($z(55) = 2.085$, $p = 0.037$, Table 1, eFigure 2). None of the endocrine measures displayed a significant group by sex interaction ($ps > 0.5$).

Tract-based DTI Analyses

Tract-based analyses were performed on seven white matter pathways, leading to a multiple comparisons threshold after Sidak correction of $p \leq 0.0073$, and were covaried for age, sex and site. Results indicated that only the UF tract significantly differed between groups (Figure 1 **&** Figure 2). Specifically, AD children had reduced UF FA compared to control children ($t(92) = 3.650$, $p < 0.001$, effect size: Cohen's d = 0.73. See also Figure 1 **&** Figure 2). In addition, the interaction between group and sex was significant for UF FA ($t(92) =$

3.058, $p = 0.003$). *Post hoc* tests indicated that AD boys displayed significantly reduced UF FA compared to control boys ($t(44) = 4.750$, $p < 0.001$, Figure 1), whereas AD girls did not differ from control girls ($t(46) = 0.547$, $p = 0.587$, Figure 1). To further investigate which of these 4 groups were the different from the rest (AD girls, AD boys, control girls, control boys), we ran a contrast analysis. Results indicated that AD boys were different from all three other groups ($t(92) = 3.937$, $p < 0.001$).

Other variables that could potentially influence the results include: age, comorbid symptoms, and hormonal status. While there was a main effect of age on UF FA ($t(92) = 2.786$, $p = 0.006$), no significant sex by age or group by sex by age interactions were found ($p > 0.28$; eTable 1 **&** eFigure 3). In relation to anxiety, depression and ADHD scores, we performed analyses to test for interactions and found that these measures did not impact the findings, such that there were no significant group by sex by rating interactions ($ps > 0.375$, eTable 1). We also performed analyses that examined potential influences of the comorbid categorical diagnoses, ADHD (4 females, 2 males) and depression (3 females, 0 males). The results remained largely unchanged when excluding these patients from the analyses (Excluding ADHD; Group: $t(86) = 3.592$, $p < 0.001$, Group x Sex: $t(86) = 2.502$, $p = 0.014$. Excluding MDD; Group: $t(89) = 3.749$, $p < 0.001$, Group x Sex: $t(89) = 2.872$, p = 0.005). Finally, we tested hormonal status (cortisol, testosterone and estradiol) in relation to the findings, which demonstrated no significant interactions that could account for the effects (Group by Sex by Hormone level; $ps > 0.175$; eTable 1).

Other diffusivity measures explored besides FA, such as MD, XD and RD indicated that only UF RD was significantly increased in AD children ($t(92) = -2.813$, $p = 0.006$: Cohen's d = 0.56, eFigure 4), and the Group by Sex interaction was nominally, uncorrected, significant ($t(92) = -2.007$, $p = 0.048$, eFigure 4). Several of the other six white matter tracts displayed some nominally, uncorrected, significant group effects and group by sex interactions for FA, MD, XD and RD (see Figure 2/eFigure 4). However, none passed multiple comparison corrections.

Voxel-based Analyses of FA

Whole-brain voxel-based analyses explored group differences beyond the a *priori* hypothesized tracts. The results confirmed the observations from the tract-based analyses, demonstrating significantly reduced FA in multiple locations along the UF tract ($p < 0.05$, TFCE corrected, Figure 1, Table 2**, and** https://neurovault.org/collections/161/). Areas particularly affected included white matter adjacent to the orbital gyrus, as well as white matter in the bend around the lateral fissure between the temporal and frontal lobes. We also detected group differences in the CC, IC and IFO pathways (Table 2 & https://neurovault.org/collections/161/). In the CC tract, alterations were mainly localized in the genu ($p < 0.05$, TFCE corrected, Table 2 **&** https://neurovault.org/collections/161/). In the left and right IC, significant clusters were found in the area of the postcentral gyrus in the somatosensory cortex ($p < 0.05$, TFCE corrected, Table 2 **&** https://neurovault.org/collections/161/). In the IFO tract significant clusters were mainly located in the anterior inferior portions of the right IFO ($p < 0.05$, TFCE corrected, Table 2 **&** https://neurovault.org/collections/161/).

Based on results from the tract-based analyses, we also examined the group by sex interaction, which did not reveal any clusters passing TFCE correction at $p = 0.05$. However, separate analyses performed in girls and boys revealed significant FA reductions in AD boys compared to control boys in regions that overlap with the UF, CC and bed nucleus of the stria terminalis, and no such differences in girls (eFigure 5 **&** eTable 2 **&** https://neurovault.org/collections/161/).

Discussion

This study examined the microstructural integrity of the UF in unmedicated, preadolescent children with ADs and also examined potential sex differences. The findings revealed decreased UF FA in AD boys, and not girls. Tractography analyses of other white matter tracts demonstrated the relative specificity of this finding. The UF is a white matter tract critical for prefrontal-temporal lobe functional integration[38], and the current data suggest that in boys this tract may be linked to the prefrontal-limbic dysregulation that is associated with ADs[20–25]. While UF FA differences have been reported in adolescents and adults with ADs, we are unaware of any reports that have addressed the issue of sexual dimorphism in AD patients. However, there have been inconsistent reports of sex related UF FA alterations in individuals with high levels of trait anxiety[39–41].

Importantly, we demonstrate that these white matter alterations are present early in life and in children that have never been exposed to psychotropic medications. In contrast to studies in adult AD populations[20], the results from this study can be interpreted without the potential confounds of prior medication exposure and/or illness chronicity. These findings provide developmental continuity in relation to the previously reported UF FA reductions in adolescents and adults, suggesting that the UF white matter alterations observed in these older populations may have their origins in childhood.

The UF FA differences that we found in AD boys are intriguing and raises the question as to what underlies this sexual dimorphism. We found no evidence that age or sex hormones could account for this finding. Previous studies in children and adults indicate either no, or a minimal, effect of sex on UF FA[42–44]. Likewise, studies in pubertal children examining sex steroids have found little association between hormonal levels and white matter volume[45,46], however little is known about the specific relation between sex hormones and UF FA. In contrast, age is well known to be associated with FA[42,43,47,48], and even with the relatively constrained age that we studied, we found that UF FA increased with age. One possibility that could provide an explanation for the sexually dimorphic finding reported here, is that the developmental trajectory of UF FA could differ between boys and girls. However, we found no significant age by sex interactions, which is consistent with other work[43,44], and importantly we found no age by sex by group interactions. We also assessed levels of the stress hormone cortisol and found no significant influences of cortisol on UF FA that could account for the UF FA reductions in boys with ADs, or interactions with sex. Because of the episodic nature of the secretion of steroid hormones, it is possible that more frequent sampling would reveal a relation between steroid hormone levels and UF FA.

Most, but not all DTI studies of AD patients report reductions in UF FA, as well as other white matter alterations[20–25,30,31,49,50]. Inconsistencies in the UF FA findings across studies could be due to a variety of factors, including heterogeneity in diagnostic composition of the sample, sample size, and DTI acquisition/processing. We also note that reduced UF FA is not specific to ADs, as similar white matter alterations have been observed in some studies of individuals with trait anxiety[39–41,51–54], as well as in patients with affective and other psychiatric disorders[55,56].

Our assessment of multiple diffusivity measures allows for a deeper understanding of the nature of the microstructural alterations found in AD children. In addition to reduced FA, AD boys exhibited increased RD. The combination of decreased FA and increased RD is thought to reflect reduced myelination and/or reduced axonal density[57,58]. Changes in myelination and axonal density are associated with alterations in the speed, timing and accuracy of information passing through white matter[59]. Because the UF is the major tract connecting medial temporal lobe structures, such as the amygdala and hippocampus, with prefrontal cortex, alterations in UF structure are likely to alter information flow relevant to emotion regulation[60]. Although lesions affecting the UF can alter the regulation of anxiety in primates[61–63], the extent to which individual differences in UF myelination influence anxiety-related neuronal signaling is unclear. Of note, a recent study in humans demonstrates that individual differences in UF FA are related to individual differences in cognitive-emotional processing[64].

Although axon myelination is most active early in development, the microstructural integrity of UF continues to increase into adulthood and is one of the last white matter pathways to reach peak FA[43]. Myelin producing oligodendrocytes are highly plastic throughout adulthood and oligodendrocyte precursor cells are the major proliferating cell type in adult brains[65]. Evidence is accumulating that myelination occurs dynamically in response to neural activity and can continue throughout adulthood[59,66,67]. This white matter plasticity provides a potential mechanism by which targeted therapies could be focused on restoring UF integrity. Interestingly, data in children suggest that aerobic exercise increases white matter integrity[68], and it is well know that exercise has antianxiety effects[69]. Our data suggests that early life interventions targeted at increasing UF integrity could be particularly effective in boys in ameliorating the symptoms of anxiety, and also could have a protective impact on the development of white matter connectivity between brain regions critical for adaptive emotion regulation.

Taken together with other studies[16–25], these data provide convergent support for PFC-temporal lobe dysfunction, that now extends to childhood anxiety. The findings demonstrate early life alterations in a key white matter tract involved in conveying information relevant to emotion and anxiety regulation. The findings also point to the importance of considering brain related sex differences prior to puberty. The data from this study, along with future studies, has the potential to guide the development of novel treatments focused on restoring the adaptive prefrontal regulation of anxiety. Such early interventions support the possibility of treating AD children with therapies that could reduce or even prevent long term chronic psychopathology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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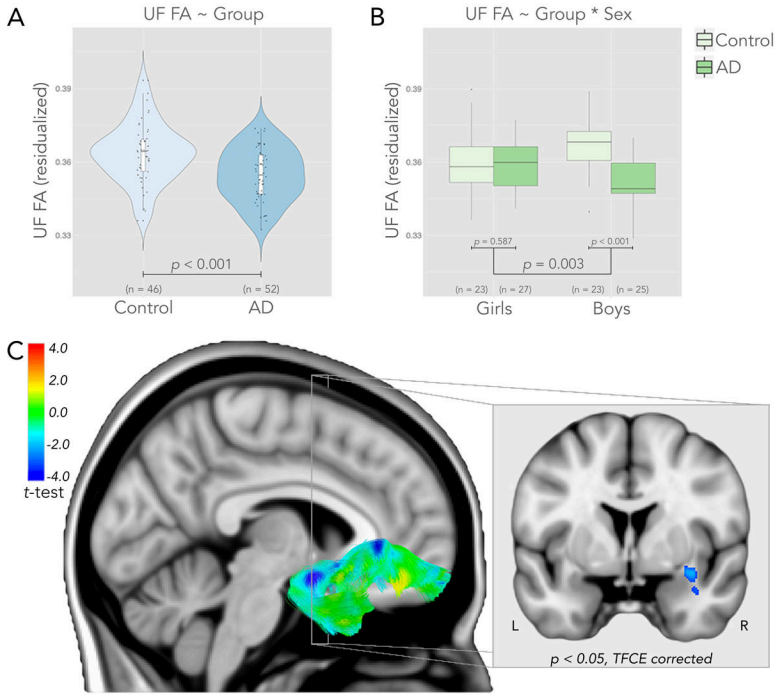
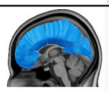
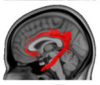
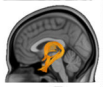
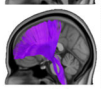
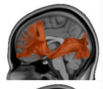
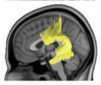
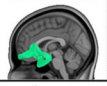


Figure 1.

Children with anxiety disorders (ADs) have reduced fractional anisotropy (FA) in the uncinate fasciculus (UF). A) Children with ADs have significantly lower FA in the UF compared to controls. Violin plot of UF FA data residualized for sex, age and site. B) Box plot of significant interaction between group and sex. *Post hoc* analyses indicate no significant difference between healthy girls and AD girls, but are different between healthy boys and AD boys. UF FA is residualized for age and site. C) Whole-brain voxel-based analyses confirm significant differences in UF FA between controls and AD children, test results are shown on the right UF fiber tract. The coronal view shows the extent of significant differences after TFCE correction. Cooler colors indicate lower FA values in AD patients compared to healthy controls.

		FA		Group	Group*Sex
Bilateral WM Tract		Control	AD	*p*-values	*p*-values
CC		0.481 *(0.013)*	0.472 *(0.017)*	0.011*	0.102
CING		0.278 *(0.014)*	0.269 *(0.015)*	0.008*	0.089
FX		0.297 *(0.016)*	0.289 *(0.017)*	0.075	0.011*
IC		0.434 *(0.011)*	0.434 *(0.014)*	0.460	0.236
IFO		0.412 *(0.017)*	0.407 *(0.018)*	0.339	0.024*
SLF		0.376 *(0.017)*	0.369 *(0.021)*	0.218	0.166
UF		0.363 *(0.013)*	0.354 *(0.013)*	**<0.001****	**0.003****

* indicates significance at uncorrected levels, ** indicates significant results at Šidák corrected levels. Abbreviations: Corpus Callosum (CC), Cingulum bundle (CING), Fornix (FX), Internal Capsule (IC), Inferior Fronto-Occipital fasciculus (IFO), Stria Terminalis (STRIA), Superior Longitudinal Fasciculus (SLF), Uncinate Fasciculus (UF).

Figure 2.

Group differences in tract fractional anisotropy (FA). Weighted mean FA values by tract for control and anxiety disorder (AD) subjects, with standard deviations in parentheses. Significance of the regression statistics for the main effect of group, as well as the interaction of group by sex are noted. All analyses include age, sex and site as covariates.

Table 1.

Demographic, symptom and hormone levels for children with anxiety disorders (ADs) and controls.

Demographics	Control	AD	*p*-values	Control Girls	AD Girls	*p*-values	Control Boys	AD Boys	*p*-values
Total; number (% female)	46 (50%)	52 (51%)	0.854	23 (100%)	27 (100%)		23 (0%)	25 (0%)	
Age (years); mean (SD)	10.58 (1.29)	10.42 (1.33)	0.701	10.74 (1.32)	10.50 (1.23)	0.633	10.38 (1.30)	10.39 (1.43)	0.987
IQ (WASI); mean (SD)	119.52 (13.44)	117.42 (13.52)	0.377	119.00 (16.33)	116.12(12.81)	0.313	120.04(10.11)	118.72(14.33)	0.696
Physical development (Tanner); mean (SD)	3.93 (2.06)	4.04 (1.87)	0.14	4.20 (2.09)	4.65 (2.00)	0.095	3.36 (1.71)	3.67 (1.86)	0.607
Symptom measure scores									
Anxiety (parent SCARED); mean (SD)	7.21 (8.83)	28.59 (12.71)	<0.001*	6.01 (6.13)	29.56(12.00)	<0.001*	6.10 (8.26)	27.78(13.15)	<0.001*
Anxiety (child SCARED); mean (SD)	13.41 (11.26)	25.93 (13.71)	<0.001*	12.44 (9.93)	31.20(13.73)	<0.001*	11.92 (9.23)	21.36(12.52)	0.005*
Depression (CDI); mean (SD)	28.68 (18.87)	38.52 (21.21)	<0.001*	27.18(18.96)	44.81(21.26)	<0.001*	27.52(17.73)	33.32(19.80)	0.010*
ADHD (CPRS-R); mean (SD)	45.67 (6.91)	61.06 (12.74)	<0.001*	46.05 (4.68)	65.40(14.24)	<0.001*	42.74 (2.68)	57.25 (9.34)	<0.001*
Endocrines Measures									
Cortisol (Saliva); mean (SD)	0.100 (0.038)	0.102 (0.039)	0.894	0.095(0.042)	0.107(0.041)	0.738	0.104(0.033)	0.095(0.036)	0.505
Testosterone (Saliva); mean (SD)	24.113(9.322)	22.628(12.330)	0.216	28.538(8.449)	25.389(14.719)	0.155	18.883(7.659)	19.176(7.579)	0.469
Estradiol (Saliva); mean (SD)	0.778 (0.313)	0.648 (0.350)	0.037*	0.788(0.377)	0.650(0.415)	0.184	0.766(0.233)	0.645(0.261)	0.205

* indicates two-tailed significance

Table 2.

Group differences (control vs. AD) in voxel-based analyses of whole-brain FA values. Overview of size and location of significant clusters after TFCE correction.

Cluster #	Volume (mm^3)	Location	Hemisphere	Peak *t*-value	Peak MNI Coordinate
1	7000	CC/UF	L & R	5.6	(88 153 73)
2	425	IC	R	4.3	(111 89 140)
3	290	UF	R	4.85	(126 127 59)
4	228	CC/IFO	R	4.53	(118 74 103)
5	148	IC	L	5.11	(74 98 133)
6	10	IFO	R	3.03	(120 148 85)
7	4	UF	R	3.47	(129 124 45)
8	3	CC	R	4.35	(111 59 121)