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Age-related abnormalities in white matter microstructure in autism spectrum disorders

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Abstract

Abnormalities in structural and functional connectivity have been reported in autism spectrum disorders (ASD) across a wide age range. However, developmental changes in white matter microstructure are poorly understood. We used a cross-sectional design to determine whether white matter abnormalities measured using diffusion tensor imaging (DTI) were present in adolescents and adults with ASD and whether age-related changes in white matter microstructure differed between ASD and typically developing (TD) individuals. Participants included 28 individuals with ASD and 33 TD controls matched on age and IQ and assessed at one time point. Widespread decreased fractional anisotropy (FA), and increased radial diffusivity (RaD) and mean diffusivity (MD) were observed in the ASD group compared to the TD group. In addition, significant group-by-age interactions were also observed in FA, RaD, and MD in all major tracts except the brain stem, indicating that age-related changes in white matter microstructure differed between the groups. We propose that white matter microstructural changes in ASD may reflect myelination and/or other structural differences including differences in axonal density/arborization. In addition, we suggest that white matter microstructural impairments may be normalizing during young adulthood in ASD. Future longitudinal studies that include a wider range of ages and more extensive clinical characterization will be critical for further uncovering the neurodevelopmental processes unfolding during this dynamic time in development.

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Keywords

autism; white matter; DTI; age; interaction

1.0 Introduction

Autism symptom presentation and severity is heterogeneous and varies throughout development. In addition to the core features of autism spectrum disorder (ASD), children and adolescents with ASD often develop maladaptive behaviors including irritability, hyperactivity, aggression, depression, and anxiety, among others (Anderson et al., 2011). During the adolescent period, individuals with ASD appear to be at a higher risk for developing seizures and behavior problems and psychiatric symptoms may increase (Gillberg and Steffenberg, 1987). Limited information is available about changes in autism symptoms from childhood to early adulthood, but emerging evidence suggests that core-ASD symptoms may be milder in adulthood than during early development (Boelte and Poustka, 2000; Gilchrist et al., 2001; Piven et al., 1996). These behavioral improvements with age may be a result of maturation and the stabilization of disease processes. However, very little is known about age-related changes in brain structure and function in the period from adolescence through adulthood in ASD.

As with manifestations of autism symptomatology, structural and functional brain abnormalities can change across the lifespan. For example, enlarged brain volume has been reported in young children with ASD (Courchesne et al., 2001; Hazlett et al., 2005; Sparks et al., 2002), particularly in early childhood, despite normal head circumference at birth (Courchesne et al., 2003; Dawson et al., 2007; Dementieva et al., 2005; Hazlett et al.). By later childhood or adolescence, brain enlargement seems to resolve (Aylward et al., 2002; Courchesne, 2004; Hardan et al., 2003; Redcay and Courchesne, 2005) and many earlier observed morphological differences are no longer apparent; however, an abundance of evidence suggests that brain function has not completely normalized. Brain imaging studies of adolescent and adult individuals with autism spectrum disorders (ASD) suggest that abnormalities in neural circuitry and connectivity are present (for review, see Williams and Minshew, 2007).

Volumetric studies of ASD have shown that some brain regions are disproportionately enlarged (Aylward et al., 2002; Courchesne et al., 2001; Hazlett et al., 2005; Kemper and Bauman, 1998; Redcay and Courchesne, 2005; Sparks et al., 2002) and grow out of synchrony with other brain regions (Hardan et al., 2006; Langen et al., 2007). Increased brain size in ASD during early development has implicated abnormalities of both grey matter (Friedman et al., 2006; Petropoulos et al., 2006) white matter, particularly in the superficial/radial white matter regions of the cerebrum (Herbert et al., 2004) and in the frontal lobes (Carper et al., 2002). Although these white matter volumetric findings do not directly support abnormal connectivity among brain structures in autism, the abnormal growth patterns are consistent with this consideration.

Functional imaging studies have provided further, indirect evidence of connectivity abnormalities in adolescents and adults with ASD. Studies utilizing functional connectivity (fcMRI) techniques have identified abnormal connectivity between brain regions involved in mediating complex language, selective attention, visuomotor coordination, emotion perception and executive functioning tasks (see, e.g. Just et al., 2004; Just et al., 2006; Kana et al., 2006; Kleinhans et al., 2008; Koshino et al., 2005; Mizuno et al., 2006; Mostofsky et al., 2009; Rudie et al., in press; Welchew et al., 2005). In most studies, under-connectivity has been reported, suggesting reduced within-network efficiency (Muller et al., 2011).

However, it is important to note that a growing literature has reported over-connectivity in ASD (see, e.g. Mizuno et al., 2006; Monk et al., 2010; Welchew et al., 2005), which may reflect inadequate synaptic pruning or other downstream effects (Muller et al., 2011).

Diffusion tensor imaging (DTI) studies provide complementary indirect evidence of abnormal white matter structural connectivity. Although most studies to date have documented widespread reductions in white matter integrity in ASD compared to controls, a few notable exceptions exist. A report of 7 children with autism between 18 and 40 months of age found increased fractional anisotropy (FA) in the genu and splenium of the corpus callosum, left posterior limb of the internal capsule, and left forceps minor (Ben Bashat et al., 2007); reduced FA was observed in the left corticospinal tracts. Similarly, in a conference presentation, Courchesne and colleagues reported increased FA in the superior longitudinal fasciculus, forceps minor, uncinate fasciculus, and the corpus callosum in children with autism between 13 and 43 months (Solso et al., 2011). In a sample with a slightly broader age-range (1.5 – 5.8 years), only the genu and body of the corpus callosum were found to retain significantly increased FA values (Weinstein et al., 2011). A recent longitudinal DTI study has largely confirmed the postulated transient nature of white matter integrity measurement in very young children with autism. Wolff et al (2012) reported increased FA in 6 month-old infants who were later diagnosed with autism in the body of the corpus callosum, left fornix, left inferior longitudinal fasciculus, right posterior limb of the internal capsule, and left uncinate. Notably, the trend in all fiber tracts except the left anterior thalamic radiation was in the direction of increased FA in the 6-month-old children with autism compared to high-risk but typically developing peers. At twelve months, although the comparisons did not reach statistical significance, the same pattern of globally increased FA was observed, still with the exception of the anterior thalamic radiation bilaterally. However, by 24 months of age, the trend reversed, with decreased FA becoming the dominant pattern across all fiber tracts. Although the available data on very young children is still limited, it seems clear that once children with autism reach 3–4 years of age, increased FA is no longer evident (but see Cheng et al., 2010; Cheung et al., 2009; Ke et al., 2009). Instead, studies of young children whose approximate age range is 2.5–9 years of age (with a mean of 5) reported reduced FA in the uncinate fasciculus, inferior fronto-occipital fasciculus, arcuate fasciculus, right cingulum, and the corpus callosum (Kumar et al., 2010), and in the short association fibers of the frontal lobe (Sundaram et al., 2008), and no regions with increased FA. The pattern of white matter abnormalities becomes more widespread with older children (aged 6–14), who show reduced FA in frontal corona radiata (Barnea-Goraly et al., 2011), corpus callosum (Barnea-Goraly et al., 2011; Brito et al., 2009), internal and external capsules (Barnea-Goraly et al., 2011; Brito et al., 2009), uncinate fasciculus (Poustka et al., in press), superior longitudinal fasciculus (Barnea-Goraly et al., 2011; Fletcher et al., 2010; Poustka et al., 2012), cingulate gyrus (Barnea-Goraly et al., 2011), temporal lobes (Barnea-Goraly et al., 2011; Cheung et al., 2009; Ke et al., 2009), parietal lobes (Barnea-Goraly et al., 2011), prefrontal white matter (Cheung et al., 2009; Ke et al., 2009) right corticospinal tract (Brito et al., 2009), and the cerebellum (Brito et al., 2009; Cheung et al., 2009). Despite a normalization of brain volume in adolescents and adults with ASD, white matter abnormalities persist. Studies of individuals in the adolescent and adult age range have reported reduced FA in the superior longitudinal fasciculus (Bloemen et al., 2010; Cheng et al., 2010; Groen et al., 2011; Jou et al., 2011; Noriuchi et al., 2010; Shukla et al., 2010), inferior longitudinal fasciculus (Bloemen et al., 2010; Groen et al., 2011; Shukla et al., 2010), left posterior limb of internal capsule (Cheng et al., 2010; Shukla et al., 2010), right inferior cerebellar peduncle (Cheng et al., 2010), corona radiata (Groen et al., 2011), corpus callosum (Bloemen et al., 2010; Jou et al., 2011; Noriuchi et al., 2010; Shukla et al., 2010), fronto-occipital fasciculus (Bloemen et al., 2010; Jou et al., 2011; Noriuchi et al., 2010; Shukla et al., 2010), left dorsolateral prefrontal cortex (Noriuchi et al., 2010), right temporal pole (Noriuchi et al., 2010), cingulum (Bloemen et al., 2010;

Shukla et al., 2010), anterior limb of internal capsule (Shukla et al., 2010), corticospinal tract (Bloemen et al., 2010; Shukla et al., 2010), anterior thalamic radiation (Bloemen et al., 2010; Shukla et al., 2010) and the uncinate fasciculus (Bloemen et al., 2010). Although fewer reports are available, mean diffusivity (MD) appears to be consistently higher in individuals with autism across various age ranges, or no different from controls. Increased MD was reported in the corpus callosum (Alexander et al., 2007; Brito et al., 2009; Groen et al., 2011; Shukla et al., 2010), corona radiata (Groen et al., 2011), anterior and posterior limb of the internal capsule (Groen et al., 2011; Shukla et al., 2010), middle cerebellar peduncle (Groen et al., 2011), thalamus and thalamic radiations (Groen et al., 2011; Shukla et al., 2010), inferior and superior longitudinal and fronto-occipital fasciculus (Groen et al., 2011; Shukla et al., 2010), temporal lobes (Lee et al., 2007), cingulum, corticospinal tract (Shukla et al., 2010), external capsule (Shukla et al., 2010), and uncinate fasciculus (Shukla et al., 2010). Two studies found no significant differences in MD (Barnea-Goraly et al., 2011; Weinstein et al., 2011).

Evidence is beginning to accrue that abnormal brain connections may underlie functional abnormalities and their concomitant behavioral abnormalities in ASD (Belmonte et al., 2004; Cherkassky et al., 2006; Courchesne and Pierce, 2005; Courchesne et al., 2007; Just et al., 2004; Muller, 2007). It has been suggested that altered levels of brain activation and underconnectivity could be secondary to abnormal development of gray matter, white matter, or both (Just et al., 2004; Just et al., 2006). A series of neuropathological studies have provided clues to the neurobiological basis of reduced connectivity including ongoing neuroinflammatory processes in the frontal lobes and cerebellum (Vargas et al., 2005) and abnormally small and densely packed minicolumns (Buxhoeveden et al., 2006; Casanova et al., 2006). Casanova and colleagues propose that abnormalities in minicolumnar development combined with larger than normal brain size contribute to neural circuit dysfunction in individuals with ASD (Casanova and Tillquist, 2008). This aberrant neurodevelopmental pattern has been hypothesized to result in abnormally increased local cortical connectivity but reduced long-distance reciprocal connectivity (Casanova et al., 2006; Casanova and Tillquist, 2008; Courchesne and Pierce, 2005). Further, a recent postmortem study of white matter in ASD reported fewer long distance axons, thinner axons, and excessive axonal branching in adults with ASD (Zikopoulos and Barbas, 2010). Overall, the growing DTI literature in ASD suggests that poor white matter integrity likely contributes to impaired communication across brain regions.

Many advances have been made in characterizing brain changes during the early childhood period, yet little is known about the pathophysiological mechanisms that contribute to ongoing, but potentially remitting or diminishing autism symptoms during adolescence and adulthood. In our current study, we used DTI to investigate white matter integrity in a cross-sectional sample of high functioning adolescents and adults with ASD, compared to typically developing (TD) controls, using a conservative, whole-brain analytic approach. In addition to characterizing differences between the two diagnostic groups, we focused on age-related changes in an effort to understand ongoing developmental processes in ASD. We hypothesized 1) that the ASD group would have reduced white matter integrity compared to an age and IQ matched TD group. Based on previous literature, we further predicted 2) that white matter integrity would show evidence of normalizing with age in the ASD group. We also hypothesized 3) that age-related rates of change would be significantly different between the TD and ASD groups.

2.0 RESULTS

2.1 Group comparison between ASD and TD

Compared with the TD group, ASD participants demonstrated widely distributed reduced white matter integrity characterized by increased MD and RaD and reduced FA values. No significant group differences were found in AxD. Regions with reduced FA included association fibers (cingulum, fornix, stria terminalis, sagittal stratum, superior fronto-occipital fasciculus, superior longitudinal fasciculus, uncinate fasciculus), brainstem tracts (inferior, middle, and superior cerebellar peduncle, medial lemniscus, and pontine crossing tract), the corpus callosum, tapetum, and all projection fibers (anterior and superior corona radiata, anterior and posterior limb and retrolenticular internal capsule, external capsule, cerebral peduncle, and corticospinal tract, posterior thalamic radiation). Increased MD was observed in the cingulum, sagittal stratum, superior longitudinal fasciculus, body and splenium of the corpus callosum, tapetum, and several projection fibers (external capsule, posterior corona radiata, posterior limb and retrolenticular part of the internal capsule, posterior thalamic radiation, and superior corona radiata). Increased RaD was observed in association fibers (cingulum, fornix, stria terminalis, sagittal stratum, superior longitudinal fasciculus, uncinate fasciculus), the corpus callosum and tapetum, and several projection fibers (anterior and posterior corona radiata, cerebral peduncle, corticospinal tract, external capsule, posterior limb and retrolenticular part of the internal capsule, posterior thalamic radiation, and middle cerebellar peduncle). See tables 2–4 for specific locations and descriptive statistics. Additional cerebral white matter regions are reported in supplementary material.

2.2 Correlations with autism severity

There were no significant positive or negative correlations found between the ADOS severity scores and FA, MD, RaD, or AxD in the ASD group ($p > .05$, corrected for multiple comparisons). However, the trends were in the expected direction. With a liberal, uncorrected threshold of $p < .05$, FA showed a negative correlation with ADOS severity in the body of the corpus callosum, MD showed a positive correlation with ADOS severity in the genu of the corpus callosum, and RaD showed a positive relationship with ADOS severity in the entire corpus callosum and the right anterior corona radiata. No statistical trend was observed between AxD and ADOS severity.

2.3 Correlations with age

In the ASD group, age was negatively associated with MD, AxD, and RaD; no significant age association was observed with FA. In the TD group, age was negatively associated with FA, MD, and AxD, and positively correlated with RaD. In the TD group, FA was found to decrease with age in several association fibers (cingulate gyrus, hippocampus, fornix, sagittal stratum, superior fronto-occipital fasciculus, and the superior longitudinal fasciculus), brain stem tracts (middle and superior cerebellar peduncle and medial lemniscus), the corpus callosum and tapetum, and several projection fibers (anterior, superior, and posterior corona radiata, internal capsule, external capsule, posterior thalamic radiation, cerebral peduncle). (See supplementary tables). Major white matter tracts in which MD decreased with age in both the ASD and TD groups included the stria terminalis, sagittal stratum, superior fronto-occipital fasciculus, uncinate fasciculus, the body and genu of the corpus callosum, the anterior and superior corona radiata, anterior and posterior limb of the internal capsule, external capsule, and the cerebellar peduncle. The ASD group showed additional regions in which MD decreased over the age-span of the evaluated subjects, including the cingulum, splenium of the corpus callosum, the tapetum, posterior corona radiata, posterior thalamic radiation, and the retrolenticular part of the internal capsule. Major white matter tracts in which AxD decreased with age in both the ASD and

TD groups included the cingulum, stria terminalis, sagittal stratum, superior fronto-occipital fasciculus, superior longitudinal fasciculus, uncinate fasciculus, the corpus callosum, the tapetum, anterior, superior and posterior corona radiata, anterior, retrolenticular, and posterior limb of internal capsule, cerebral peduncle, external capsule, and posterior thalamic radiation. There were several additional major tracts that showed a negative correlation between AxD and age in the TD group only, including the middle, inferior, and superior cerebellar peduncle, pontine crossing tract, and medial lemniscus, and the corticospinal tract. The ASD group showed a negative age relationship while the TD group showed a positive age relationship with RaD for a number of tracts, including the stria terminalis, sagittal stratum, superior longitudinal fasciculus, the body and splenium of the corpus callosum, and the cerebral peduncle. Notably, the ASD group showed an age-related decrease in RaD in the cingulum, uncinate fasciculus, genu of the corpus callosum, tapetum, anterior corona radiata, and anterior limb of internal capsule whereas no significant age effects were detected for these regions in the TD group.

2.4 Diagnostic group by age interaction

Significant diagnostic group by age interaction effects were observed in FA, MD, and RaD as shown in Table 5. FA values generally decreased in the TD group and slightly increased in the ASD group as a function of age in the cingulate gyrus, sagittal stratum, the corpus callosum, tapetum, corona radiata, internal capsule, external capsule, cerebral peduncle, and the posterior thalamic radiation. The significant interaction term in MD reflected a positive age correlation in the TD group and a negative age correlation in the ASD group in the cingulum, superior longitudinal fasciculus, the body and splenium of the corpus callosum, the posterior thalamic radiation, and the posterior and superior corona radiata. Similarly, the significant interaction term in RaD reflected a positive age correlation in the TD group and a negative age correlation in the ASD group in the following regions: cingulum, stria terminalis, sagittal stratum, superior longitudinal fasciculus, the corpus callosum, tapetum, corona radiata, cerebral peduncle, external capsule, internal capsule, and the posterior thalamic radiation. No group by age interaction was found in AxD.

3.0 DISCUSSION

Our study demonstrated that white matter structural integrity is altered across all major tracts in high functioning adolescents and adults with ASD compared to age and IQ matched TD individuals. The major findings were reduced FA and increased RaD in the ASD group, as well as a number of age-related interactional effects. This work adds to a limited number of studies that have investigated white matter integrity in ASD, and demonstrates convincingly that white matter microstructure is atypical in ASD and appears to follow an abnormal developmental trajectory during adolescence and adulthood.

Due to the vast number of affected areas, we focused on the major white matter tracts implicated in ASD and detailed in the John's Hopkins University (JHU) white matter atlas (Mori et al., 2005). Additional areas that showed statistically significant results are reported in the supplementary information section. The JHU atlas classifies white matter tracts into four groups. The projection tracts connect cortical and subcortical grey matter, the association tracts connect cortical areas, the commissural tracts connect the left and right hemispheres, and the brain stem tracts are the five major white matter tracts that can be reconstructed in the brainstem (Mori et al., 2005). We found reduced FA in the ASD group in all white matter tracts included in the JHU atlas with the exception of the bilateral hippocampus tracts (association fibers), the right inferior cerebellar peduncle (brain stem tract), and the left tapetum (commissural fibers). The majority of white matter tracts with reduced FA also had corresponding increases in RaD. Our study is consistent with most previous DTI studies of children and adults with ASD, although we found considerably

more widespread abnormalities than had been previously reported. The most consistently reported findings in the extant literature have been abnormal FA in the cingulate gyrus (Barnea-Goraly et al., 2011; Bloemen et al., 2010; Jou et al., 2011; Pardini et al., 2009; Shukla et al., 2010; Thakkar et al., 2008; Weinstein et al., 2011), inferior fronto-occipital fasciculus (Bloemen et al., 2010; Cheng et al., 2010; Jou et al., 2011; Noriuchi et al., 2010; Shukla et al., 2010), superior longitudinal fasciculus (Barnea-Goraly et al., 2011; Bloemen et al., 2010; Cheng et al., 2010; Cheung et al., 2009; Jou et al., 2011; Noriuchi et al., 2010; Poustka et al., in press; Shukla et al., 2010), the corpus callosum (Barnea-Goraly et al., 2011; Ben Bashat et al., 2007; Bloemen et al., 2010; Brito et al., 2009; Cheon et al., 2011; Kumar et al., 2010; Noriuchi et al., 2010; Shukla et al., 2010; Weinstein et al., 2011), and the posterior limb of the internal capsule (Barnea-Goraly et al., 2011; Ben Bashat et al., 2007; Brito et al., 2009; Cheng et al., 2010; Shukla et al., 2010). A few studies (Bloemen et al., 2010; Cheon et al., 2011; Kumar et al., 2010; Poustka et al., in press) in addition to ours have found reduced FA in the uncinate fasciculus, the tract that connects the orbital aspect of the frontal lobe to the temporal pole and terminates in the amygdala. The uncinate fasciculus is thought to be involved in processing information about the emotional significance of stimuli and the generation of emotional expression (Schmahmann and Pandya, 2006). However, white matter abnormalities in ASD are widespread, affecting all major neural systems. See Table 6 for a summary of FA in autism, organized according to the age of the sample.

FA is a measure of the ratio of restricted diffusion to restrained diffusion and is thought to be related to intracellular diffusion along axonal microtubules, diffusion constrained to the myelin-cell membrane border or diffusion directed by the outer sheath of the myelin complex (Song et al., 2005). Reduced FA indicates potential abnormalities in white matter tissue integrity and has been reported in several neurological diseases such as multiple sclerosis, Parkinson's disease, Alzheimer's disease, Huntington's disease and traumatic brain injury (see e.g., Weaver et al., 2009). RaD is a measure of diffusion perpendicular to the long axis of the white matter tract. Although controversy exists as to how this measure should be interpreted (Wheeler-Kingshott and Cercignani, 2009), evidence suggests that changes in RaD are associated with the process of myelination and demyelination, as opposed to axonal degeneration (Song et al., 2005). One report showed strong correlations between histological markers of axonal tissue and AxD diffusivity values in an animal model of spinal cord injury and between myelin concentrations and RaD diffusivity values in an animal model of multiple sclerosis (Budde et al., 2007). The combined evidence stemming from this and other animal work suggests that diffusivity values can differentiate between axonal damage and myelin loss. Specifically, the loss of axons within white matter decreases AxD values but does not affect RaD, while the loss of myelin increases RaD values but does not affect AxD values (Deboy et al., 2007; Harms et al., 2006; Song et al., 2002). However, it is important to exhibit caution in interpreting changes in RaD and AxD, because when crossing fibers are present, changes in RaD can be confused with changes in AxD and vice-a-versa (Wheeler-Kingshott and Cercignani, 2009).

In neurodevelopmental disorders such as ASD, interpreting group differences in DTI scalars presents additional challenges. Unlike in degenerative diseases or when a loss of white matter integrity is secondary to aging, in ASD white matter is expected to have formed abnormally and be subjected to unknown, ongoing developmental processes. Volumetric studies have shown that white matter development is atypical in ASD. Young children with ASD appear to have excessive white matter (Carper et al., 2002; Hazlett et al., 2005; Herbert et al., 2004) and, potentially, increased FA values (Ben Bashat et al., 2007; Weinstein et al., 2011; Wolff et al., 2012). However, morphological differences disappear by adolescence and adulthood while impairment is still detectable in the white matter microstructure. The mechanism for the transient, exuberant growth followed by a premature plateau (in gross

morphology) is unknown. Furthermore, direct evidence of white matter microstructural abnormalities is extremely limited. However, recently, Zikopoulos and Barbas (2010) undertook a post mortem investigation of the fine structure of myelinated axons in the prefrontal cortex of five adults with ASD aged 30–44. They found a decrease in the number of long distance axons in the white matter and an excessive number and higher density of short and medium range axons under the anterior cingulate cortex. Further analysis of this area revealed that increased density was due to increased branching along the nodes of Ranvier in the superficial white matter. In the white matter below the orbital frontal cortex there was decreased myelin thickness, independent of axonal diameter. Although this work was based on a very small sample size, our findings of abnormalities in RaD, but not AxD, would be consistent with postmortem evidence of abnormal myelin rather than axonal injury. Reduced FA, as well as increased RaD values, would be expected in the presence of reduced long distance axons with increased branching and thinner myelin. Thus, at this time, it does not appear that white matter abnormalities in ASD can be attributed to frank demyelination.

A major goal of this study was to look at age-related differences in DTI from adolescence through adulthood in ASD. Although white matter microstructural abnormalities in ASD have been discussed previously, the developmental trajectory of such abnormalities has been largely understudied. We found a number of significant age-by-diagnosis interaction effects for the association fibers, commissural fibers, and projection fibers. In general, FA, MD, and RaD levels appeared to be normalizing to TD levels over time in the ASD group. During adolescence, differences in the DTI scalars are pronounced and indicate robust differences in white matter microstructure. However, during early adulthood, the ASD group's FA, MD, and RaD values were similar to and heading in the direction that would suggest equivalent white matter integrity as the TD group. Because of the limited number of older participants that we have in this study and the limitations inherent in interpreting DTI data, it is premature to suggest that white matter integrity is improving in the ASD group over this age range. However, the age-related pattern identified in this study is not consistent with accelerated aging processes which affect myelin integrity. Future studies that look at age effects in a across a broader span of older subjects may be able to more specifically characterize the developmental processes occurring in ASD.

Age by diagnosis interaction effects were widespread throughout the white matter, yet notably absent in the brain stem tracts. In addition, the ASD groups did not show correlations between age and white matter integrity in any of the brain stem tracts. This is in contrast to the TD group, for whom age was negatively correlated with FA and AxD in the middle and superior cerebellar peduncles and the medial lemniscus. A negative correlation with AxD only was observed in the pontine crossing tract and inferior cerebellar peduncle. While the ASD group showed microstructural abnormalities in the brainstem, consistent with myelin abnormalities, such processes appear to be static in the ASD brain throughout adolescence and early adulthood. This is in stark contrast to the TD group, wherein regional declines in measures of white matter integrity were observed.

There are several limitations to consider when evaluating the current study. We sought to investigate white matter microstructural abnormalities in ASD and their relationship to age. Although we report our results in terms of the white matter tracts, it is important to keep in mind that the TBSS is a voxelwise approach, not a tractography approach, and the overlap with tracts is inferred based on the JHU atlas template. Also, because of our coarse spatial resolution (relative to axonal diameter), one voxel may contain multiple white matter tracts. Second, our ASD sample was comprised almost exclusively of high functioning male adolescents and young adults. Thus, it is not certain whether the findings reported here are generalizable to the entire spectrum of clinical presentations, levels of functioning, females,

and age groups. It is quite possible that younger and lower functioning individuals with ASD may have a different developmental course and/or level of white matter abnormalities. Third, because this was a cross-sectional study, we cannot rule out the role of cohort effects on our findings. However, our results suggest that follow-up longitudinal studies of white matter integrity are warranted in this age range. In addition, future studies should consider the role of interaction effects when statistically controlling for age when comparing ASD and TD groups. Our study indicates that investigating age as an independent variable may yield important information about the pathophysiology of ASD.

In conclusion, our study found widespread white matter abnormalities in high functioning adolescents in ASD across all major white matter tracts. Since differences were primarily in RaD, not AxD, we proposed that white matter abnormalities in ASD may be related to myelin dysfunction. In addition, increased RaD may reflect differences in axonal density and/or arborization. Although this current study does not support the presence of axonal thinning as reported in post-mortem work (Zikopoulos and Barbas, 2010), higher resolution techniques such as High Angular Resolution Diffusion Imaging, which produced modeling of crossing fibers may be a more sensitive approach for detecting axonal pathology. Dramatic developmental changes in white matter appear to be occurring during the transition period between adolescence and adulthood, although the trajectory of this change is significantly different between the ASD and TD groups. It is notable that impairments are most pronounced in adolescence, a period of time that may be characterized by increases in comorbid psychiatric difficulties and, in some cases, the onset of seizures. From adolescence to adulthood, white matter scalars appear to normalize to levels that overlap with typical developing peers. It is possible that the apparent improvements in autism symptom expression (and/or maladaptive behaviors) during adulthood are associated with improvements in white matter integrity. Additional studies utilizing a longitudinal design that includes a wider range of ages and more extensive clinical characterization would be useful for further exploration of the neurodevelopmental processes unfolding during this dynamic time in development.

4.0 Experimental Procedure

4.1 participants

Twenty-eight individuals with ASD and 33 TD controls participated in the DTI study. Data from five TD participants were excluded due to an incidental finding on MRI ($n=1$), a clinically significant elevation (moderate to severe range) on our social anxiety measure ($n=1$), or excessive artifacts ($n=3$). Data from three individuals with ASD were excluded for excessive artifacts ($n=2$) or because of distortions caused by the participant's braces ($n=1$). All participants were able to tolerate the MRI scanning protocol without sedation. The included ASD group ($n=25$; females = 9; mean age = 21.29 ± 5.66 ; range = 13.72 – 35.59) was composed of 11 individuals with autistic disorder, 9 individuals with Asperger's disorder, and 5 individuals with pervasive developmental disorder-not otherwise specified (PDD-NOS) based on expert clinical judgment utilizing DSM-IV criteria (American Psychiatric Association, 1994). Diagnoses were confirmed with the Autism Diagnostic Interview-Revised (ADI-R, Lord et al., 1994) and the Autism Diagnostic Observation Schedule (ADOS, Lord et al., 2000). All participants under 18 years of age are part of an ongoing longitudinal study at the University of Washington Autism Center and received the ADI-R and ADOS at ages 3–4, 6, 9, and 14 years of age. Adult ASD participants were administered the ADOS as part of their current research visit. Most adults were administered ADI-R as part of the current research visit; however, when a prior ADI-R was available, the ADI-R was not repeated in order to minimize participant and family burden. TD participants ($n=28$; female = 6; mean age = 21.31 ± 7.269 ; range = 13.58 – 40.92) were screened for current or past psychiatric disorders, history of a developmental learning

disability, and contraindications to MR imaging. The ASD and control groups did not significantly differ on age ($t = -.011$; $df = 51$, $p = .992$) or full-scale IQ ($t = -.787$; $df = 51$, $p = .435$). Clinical and demographic information is reported in table 1.

This study was approved by the University of Washington Human Subjects Institutional Review Board and written, informed consent was obtained from all study participants.

4.2 Data Acquisition

MRI scans were collected on a 3T Philips Achieva MR system (version 1.5, Philips Medical Systems, Best, The Netherlands) with dual Quasar gradients (80mTm⁻¹ with a slew rate of 110mTms⁻¹ or 40mTm⁻¹ at a slew rate of 220mTms⁻¹) using an 8-channel SENSE head coil. A T1-weighted MPRAGE (magnetization prepared-rapid gradient echo; TR=7.7 ms; TE=3.7 ms; flip angle = 8; FOV=220 mm; matrix 200×200; 180 slices; acquisition voxel size (mm) = 1.00/1.00/1.00; reconstruction voxel size (mm) 0.86/0.86/1.00; TFE shots=144; TFE durations=1633.0; Inversion delay (TI) 823.8 ms; slice orientation axial, fold-over direction RL; REST slab 57.1 mm slice thickness) volume was collected for registration and anatomical localization.

The DTI scan consisted of a single-shot echo-planar sequence with the following parameters: TR/TE/flip angle: 10.5 s/63 ms/90°, matrix size of 128×128, FoV of 240×240, 2 mm slice thickness, 72 slices. Diffusion weighting consisted of 32 non-colinear gradient directions, a non-diffusion weighted b0 map and a b-factor set at 1000 s/mm². The B0 field map was acquired using a fast field echo sequence (TR=200 ms; TE1=4.6 ms; TE2 = 5.6 ms; flip angle=30°; FOV=220 mm) with a matrix size of 64 × 64 (in-plane resolution=3.44×3.44 mm). Thirty-eight axial slices covering the entire brain (slice thickness = 3.5mm, 0 mm gap) were acquired during each image. Scan duration = 53 s. The B0 field map was reconstructed by subtracting the phase images from the two TE image acquisitions. The output contained a magnitude map and a B0 map.

4.3 DTI Processing and Statistical Analysis

All DTI data were preprocessed offline using FDT (fMRIB's Diffusion Toolbox; <http://www.fmrib.ox.ac.uk/fsl/fdt/index.html>). The raw DTI images were visually inspected following eddy current correction by a rater blinded to diagnosis in order to identify DTI studies that had artifacts, including “venetian blinds,” “checkers,” large intensity differences in any of the slices, wrapping, or motion artifacts (as per above, 2 ASD and 3 TD studies were excluded on this basis). Studies containing any of the artifacts on more than 10 gradient directions were excluded. For DTI studies with 10 or fewer artifact-contaminated gradient directions, the bad gradient directions were removed from the dataset and the bvec file was modified to reflect these changes. In our sample, an average of 1.7 (SD=2.2) directions were removed from the scans of the ASD group and 2.1 (SD = 3.3) directions were removed from the scans of the TD group ($p = .632$). Head motion and eddy current correction was conducted with affine registration to a reference volume. Using the field maps, B0-field inhomogeneity-induced geometric distortion was then corrected with PRELUDE (Phase Region Expanding Labeller for Unwrapping Discrete Estimates; [78]) and FUGUE (fMRIB's Utility for Geometrically Unwarping EPIs; <http://www.fmrib.ox.ac.uk.offcampus.lib.washington.edu/fsl/fugue/>). Diffusion tensors were estimated at each voxel using FDT. From these maps, λ_1 , λ_2 and λ_3 , mean diffusivity (MD), and fractional anisotropy (FA) indices were calculated. In addition, axial diffusivity (AxD; the magnitude of the primary eigenvalue) and radial diffusivity (RaD; the mean of the two eigenvalues that describe width and depth) maps were computed. Tract Based Spatial Statistics (TBSS) was used to delineate the white matter tracts and warp individual maps to the FMRIB58_FA standard-space image. Voxelwise statistics were performed using

Randomise with threshold-free cluster-enhancement (TFCE, Salimi-Khorshidi et al., 2011) using 5000 permutations. Between-group contrasts were applied to each DTI scalar map (FA, MD, RaD, AxD) independently, controlling for gender. In addition, we investigated the relationship between age and the DTI scalars for the ASD and TD group and tested the group by age interaction, controlling for IQ and gender. As a follow-up to the interaction model, we ran a correlational analyses between age and the DTI indices for the ASD and TD groups separately, controlling for IQ and gender. Lastly, in the ASD group, we tested the relationship between ADOS severity and all DTI scalars, controlling for gender and IQ. Significance for all analyses was set at $p < 0.05$, whole brain family-wise-error-corrected.

We identified affected white matter structures using the John's Hopkins University (JHU) ICBM-DTI-81 white matter atlas (Mori et al., 2005) which parcellates the brain into 50 white matter tracts. Using software developed in our laboratory, each voxel in the corrected statistical map, thresholded at $p < .05$, was assigned to a JHU tract. For each tract, the total number of significant voxels was calculated and the voxel with the highest p -value and its corresponding MNI coordinates was identified. These summary statistics are reported in the Results tables. For completeness, we also used the Talairach atlas to label white matter not included in the JHU atlas. The results of the white matter regions outside the JHU atlas are reported in the supplementary information section.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

Widespread, robust white matter microstructural abnormalities are present in ASD.

Impairments are observed in FA, MD, and radial diffusivity in ASD.

Abnormal myelination, excessive branching, or thinning may be present in ASD.

Age-by-Dx interaction effects suggest normalization may occur in adults with ASD.

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Figure 1.

Regions with significant between-group difference in FA (A.), MD (B.) and RaD(C). The TBSS skeleton is shown in green over the FA template brain. Areas in red indicate white matter regions where FA values were significantly lower in the ASD group compared to the TD group. The purple areas indicate white matter regions where MD values were significantly higher in the ASD group compared to the TD group. The blue areas indicate white matter regions where the RaD values were significantly higher in the ASD group compared to the TD group. Regions are labeled according the JHU atlas, using standard abbreviations. Additional statistical information is available in Tables 2–4.

Figure 2.

Regions with a significant interaction effect between age and diagnosis for FA (A), MD (B), and RaD (C) along with scatter plots to illustrate the directionality of the interaction effect. Each DTI scalar is overlaid on the slices that were the most representative of the results ($x = -28, y = 27, z = 23$). Red areas indicate voxels where FA values showed a significant interaction effect. Purple areas indicate voxels where MD values showed a significant interaction effect. Blue areas indicate voxels where RaD showed a significant interaction effect. Scatter plots were created by computing a mean DTI scalar value for each participant, which was obtained by averaging the z-score of all voxels for that participant included in the mask. The mask was defined by the voxels showing the significant interaction effect. The

top scatter plot is FA, the middle scatter plot is MD, and the bottom scatter plot is RaD. Additional statistical information is provided in Table 5.

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Table 1

Included participant characteristics

	ASD (n=25)		TD (n = 28)		p value
	Mean	SD	Mean	SD	
Age	21.29	(5.66)	21.31	(7.27)	.99
Full Scale IQ ^a	109.88	(16.94)	113.25	(14.24)	.44
Verbal IQ ^a	106.16	(21.00)	111.14	(13.78)	.31
Nonverbal IQ ^a	110.72	(14.25)	111.96	(14.39)	.75
ADOS subscales					
Communication	4.08	(1.96)			
Social	7.36	(2.33)			
ADOS severity	6.44	(1.39)			
ADI-R subscales					
Communication	15.36	(5.24)			
Social	19.76	(6.25)			
Repetitive Behavior	5.56	(2.33)			

^aBased on Differential Abilities Scale for participants age 13–17 and the WASI for participants age 18–40

ADOS = Autism Diagnostic Observation Schedule; ADI-R = Autism Diagnostic Interview-Revised

Table 2

Regions showing decreased fractional anisotropy in ASD

White Matter Region	side	p (max)	x	y	z	# of voxels
<u>JHU Atlas labels</u>						
Association Fibers						
Cingulum (cingulate gyrus)	L,R	.001	-10	-24	32	402
Fornix	I	.005	0	-1	14	97
Fornix / Stria terminalis	R,L	.004	29	-28	-5	351
Sagittal stratum	R,L	.002	40	-37	-12	637
Superior fronto-occipital fasciculus	L,R	.007	-21	-3	19	25
Superior longitudinal fasciculus	R,L	.001	37	-56	15	1661
Uncinate fasciculus	R,L	.004	35	-1	-12	72
Brainstem Tract						
Middle cerebellar peduncle	I	.028	5	-19	-31	232
Pontine crossing tract	I	.037	-7	-31	-28	150
Medial lemniscus	L,R	.037	-4	-34	-27	148
Superior cerebellar peduncle	L,R	.037	-7	-34	-23	194
Commissural Fibers						
Body of corpus callosum	I	.001	-13	-30	29	2441
Genu of corpus callosum	I	.003	6	24	13	1267
Splenium of corpus callosum	I	.001	-18	-39	27	2029
Tapetum	R	.005	33	-42	8	23
Projection Fibers						
Anterior corona radiata	L,R	.003	-15	37	2	1420
Anterior limb of internal capsule	L,R	.004	23	23	2	379
Cerebral peduncle	L,R	.003	17	-20	-12	510
Corticospinal tract	L,R	.025	6	-23	-32	436
External capsule	L,R	.002	-29	-19	13	1004
Posterior corona radiata	L,R	.001	-20	-37	32	917
Posterior limb of internal capsule	L,R	.001	-27	-25	18	925

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White Matter Region	MINI coordinates				
	side	p (max)	x	y	z
Posterior thalamic radiation	L,R	.001	35	-58	1
Retrolenticular part of internal capsule	L,R	.001	-34	-38	10
Superior corona radiata	L,R	.001	-21	-31	41
					662

Note. R = right, L = left, I = interhemispheric. Regions are labeled according to the peak p value within that region. When both R and L are listed, the bolded side indicates the peak p value and location, the # of voxels includes both sides combined.

Table 3

Regions showing increased mean diffusivity in ASD

White Matter Region	side	p (max)	MNI coordinates				# of voxels
<u>JHU Atlas labels</u>			x	y	z		
Association Fibers							
Cingulum (cingulate gyrus)	L,R	0.0184	11	-47	26		76
Sagittal stratum	L,R	0.035	36	-53	-4		24
Superior longitudinal fasciculus	L,R	0.0172	34	-29	35		775
Commissural Fibers							
Body of corpus callosum	I	0.0176	13	-28	28		410
Splenium of corpus callosum	I	0.0168	26	-54	14		768
Tapetum	R	0.0178	29	-50	18		3
Projection Fibers							
External capsule	L	0.0408	-30	-17	13		18
Posterior corona radiata	L,R	0.0168	28	-57	20		911
Posterior limb of internal capsule	L	0.0374	-27	-25	18		41
Posterior thalamic radiation	L,R	0.0172	29	-57	18		577
Retrolenticular part of internal capsule	L,R	0.0184	31	-38	16		158
Superior corona radiata	L,R	0.0176	25	-24	31		566

Note. R = right, L = left, I = interhemispheric. Regions are labeled according to the peak p value within that region. When both R and L are listed, the bolded side indicates the peak p value and location, the # of voxels includes both sides combined.

Table 4

Regions showing increased radial diffusivity in ASD

White Matter Region	side	p (max)	MNI coordinates			# of voxels
<u>JHU atlas labels</u>			x	y	z	
Association_Fibers						
Cingulum (cingulate gyrus)	L,R	.001	12	-47	25	308
Cingulum (hippocampus)	R	.040	25	-35	-10	45
Fornix / Stria terminalis	R	.014	35	-14	-13	29
Sagittal stratum	L,R	.001	-39	-44	-8	365
Superior longitudinal fasciculus	L,R	.001	-43	-50	2	1746
Uncinate fasciculus	L,R	.009	-34	-2	-21	30
Commissural_Fibers						
Body of corpus callosum	I	.001	-14	-30	29	2060
Genu of corpus callosum	I	.004	6	24	13	687
Splenium of corpus callosum	I	.001	-18	-40	26	1844
Tapetum	R	.004	32	-42	12	12
Projection_Fibers						
Anterior corona radiata	L,R	.004	18	17	29	913
Anterior limb of internal capsule	R	.040	22	22	1	5
External capsule	L,R	.001	-28	-21	15	376
Posterior corona radiata	L,R	.001	-20	-38	32	1138
Posterior limb of internal capsule	L,R	.001	-24	-21	8	558
Posterior thalamic radiation	L,R	.001	35	-64	-3	1696
Retrolenticular part of internal capsule	L,R	.001	-40	-35	-1	663
Superior corona radiata	L,R	.001	21	-32	40	973

Note. R = right, L = left, I = interhemispheric. Regions are labeled according to the peak p value within that region. When both R and L are listed, the bolded side indicates the peak p value and location, the # of voxels includes both sides combined.

Table 5

Regions showing a significant group by age interaction effects

White Matter Region	side	p (max)	x	y	z	# of voxels
Age by Dx interaction in FA						
Association Fibers						
Cingulum (cingulate gyrus)	L,R	.021	12	-46	25	117
Sagittal stratum	L,R	.012	-40	-34	-13	255
Superior longitudinal fasciculus	L,R	.012	-42	-47	2	454
Commissural_Fibers						
Body of corpus callosum	I	.011	-12	-30	27	910
Genu of corpus callosum	I	.022	-16	22	24	171
Splenium of corpus callosum	I	.011	-17	-41	25	838
Tapetum	L,R	.029	-28	-53	17	11
Projection_Fibers						
Anterior corona radiata	L,R	.017	-17	40	-4	426
Anterior limb of internal capsule	L	.029	-11	9	-4	23
Cerebral peduncle	L	.044	-16	-13	-7	25
External capsule	L,R	.011	-28	-22	18	38
Posterior corona radiata	L,R	.010	-27	-27	19	876
Posterior limb of internal capsule	L,R	.010	-27	-25	17	364
Posterior thalamic radiation	L,R	.011	-38	-47	1	861
Retrolenticular part of internal capsule	L,R	.011	-27	-28	18	421
Superior corona radiata	L,R	.010	-25	-23	20	615
Age by Dx interaction in MD						
Association Fibers						
Cingulum (cingulate gyrus)	L	.028	-10	-48	25	29
Superior longitudinal fasciculus	L	.022	-34	-40	32	228
Commissural_Fibers						
Body of corpus callosum	I	.028	-16	-30	31	37
Splenium of corpus callosum	I	.020	-18	-37	31	133

White Matter Region	side	p (max)	x	y	z	# of voxels
Projection Fibers						
Posterior corona radiata	L	.020	-28	-61	19	216
Posterior thalamic radiation	L	.020	-27	-60	17	215
Superior corona radiata	L	.022	-20	-29	40	117
Age by Dx interaction in RaD						
Association Fibers						
Cingulum (cingulate gyrus)	L,R	.005	-11	-34	35	225
Fornix / Stria terminalis	L,R	.007	-32	-22	-8	119
Sagittal stratum	L,R	.007	-40	-34	-14	371
Superior longitudinal fasciculus	L,R	.005	-37	-40	19	601
Commissural Fibers						
Body of corpus callosum	I	.005	-15	-30	30	796
Genu of corpus callosum	I	.024	17	20	25	1
Splenium of corpus callosum	I	.004	-17	-35	29	783
Tapetum	L,R	.011	28	-39	20	10
Projection Fibers						
Anterior corona radiata	R	.016	19	16	32	77
Cerebral peduncle	L	.037	-17	-23	-5	1
External capsule	L,R	.005	-28	-22	18	160
Posterior corona radiata	L,R	.004	-20	-36	38	1028
Posterior limb of internal capsule	L,R	.005	-27	-26	17	269
Posterior thalamic radiation	L,R	.004	-29	-70	11	1016
Retrolenticular part of internal capsule	L,R	.005	-28	-33	14	633
Superior corona radiata	L,R	.004	-19	-27	37	760

Table 6

Summary of FA results in DTI studies of ASD, organized by age range.

Study	Method	Diagnosis	Group size		Group Age, mean (SD) yr		Results	
			Autism	Control	Autism	Control	FA	Location
Wolff et al. 2012	Tractography	Autism	28	64	0.57 (0.07)	0.56 (0.07)	↑	l-fx, l-iff, l-unc, body of the cc, r-plie
Wolff et al. 2012	Tractography	Autism	17	49	1.06 (0.06)	1.06 (0.05)	↓	l-atr
Wolff et al. 2012	Tractography	Autism	17	33	2.04 (0.05)	2.06 (0.07)	↓	l-alic, l-atr
Bashat et al. 2007	ROI	Autism	7	18	1.80 – 3.30*	9.60**	↑↓	Increase: gcc, sec, l-plie, l-fminor Decrease: l-est-sl2
Weinstein et al. 2011	Whole-brain	Autism	21	26	3.30 (1.10)	3.30 (1.20)	↑	gcc, body of the cc, l-slf, cg
Sundaram et al. 2008	Tractography	Autism, PDD-NOS, Asperger's disorder	50	16	4.79 (2.43)	6.84 (3.45)	↓	short association fibers
Kumar et al. 2010	ROI	Autism, PDD-NOS, Asperger's disorder	32	16	5.00**	4.60**	↓	r-unc, l-slf, r-cg, cc
Sivaswamy et al. 2010	ROI	Autism, PDD-NOS, Asperger's disorder	27	16	5.00**	5.90**	↑	r-mcp
Ke et al. 2009	Whole-brain	Autism	12	10	8.75 (2.26)	9.40 (2.07)	↑↓	Increase: r-middle temporal gyrus, r-sub-gyral frontal lobe, l-sub-lobar Decrease: l-middle frontal gyrus, l-stg, l-inferior frontal gyrus
Cheung et al. 2009	Whole-brain	Autism	13	14	9.30 (2.60)	9.90 (2.50)	↑↓	Increase: r-slf, l-fmajor Decrease: l-frontal orbital cortex BA47, r-precentral gyrus BA4, frontal pole BA11, r-fusiform gyrus BA19, r-unc, l-middle temporal gyrus BA20
Brito et al. 2009	ROI	Autism	8	8	9.53 (1.83)	9.57 (1.36)	↓	anterior body of the cc, r-est, r-plie, l-scp, mcp
Poustka et al. in press	ROI and tractography	ASD	18	18	9.70 (2.10)	9.70 (1.90)	↓	r-slf, unc
Bamea-Goraly et al. 2011	Whole-brain	ASD	13	11	10.50 (2.00)	9.60 (2.10)	↓	medial prefrontal white matter, act, gcc, anterior forceps of the cc, body of the cc, ec, slf, mid/posterior cingulate gyrus, stg, temporo-parietal junction, fronto-parietal centrum semiovale
Jou et al. 2011	Whole-brain	ASD	15	8	10.90 (3.70)	11.50 (2.60)	↓	cg, ifo, ilf, slf, unc, atr, est, fminor
Cheon et al. 2011	ROI	Asperger's disorder, PDD-NOS	17	17	11.00 (2.10)	10.20 (2.00)	↓	atr, cc, l-unc, ilf
Ameis et al. in press	Whole-brain	Autism, Asperger's disorder	19	16	12.40 (3.10)	12.30 (3.60)	ns	whole brain
Shukla et al. 2010	Whole-brain	Autism, Asperger's disorder	26	24	12.80 (0.60)	13.00 (0.60)	↓	ilf, ifo, slf, cg, gcc, body of the cc, sec, plie, alic, est, atr
Jou et al. 2011	ROI	Autism, PDD-NOS, Asperger's disorder	10	10	13.06 (3.85)	13.94 (4.23)	↓	anterior radiation of the cc, cg, body of the cc, l-slf, l-ifo, ilf

Study	Method	Diagnosis	Group size		Group Age, mean (SD) yr		Results	
			Autism	Control	Autism	Control	FA	Location
Cheng et al. 2010	Whole-brain	ASD	25	25	13.71 (2.54)	13.51 (2.20)	↑↓	Increase: r-slf, r-scr, l-insula, r-atr, r-plie, r-ifo, mcp Decrease: r-slf, l-plie, r-icp
Noriuchi et al. 2010	Whole-brain	ASD	7	7	13.96 (2.68)	13.36 (2.74)	↓	white matter around the r-anterior cingulate cortex, l-dorsolateral prefrontal cortex, r-temporal pole, r-amygdala, r-slf, l-posterior superior-temporal sulcus, anterior cc, r-fronto-occipital fasciculus
Fletcher et al. 2010	ROI	High-functioning autism	10	10	14.25 (1.92)	13.36 (1.34)	ns	slf
Groen et al. 2011	Whole-brain	Autism	17	25	14.40 (1.60)	15.50 (1.80)	↓	slf, ilf, l-corona radiata
Bode et al. 2011	Whole-brain	ASD	27	26	14.70 (1.60)	14.50 (1.50)	↑	opt, r-ifo
Lee et al. 2007	ROI	Autism, PDD-NOS	43	34	16.20 (6.70)	16.40 (6.00)	↓	white matter of the sig, temporal stem
Alexander et al. 2007	ROI	Autism, PDD-NOS, Asperger's disorder	43	34	16.23 (6.70)	16.44 (5.97)	↓	gcc, sec, total cc
Knaus et al. 2010	tractography	ASD	7	15	16.83 (2.35)	14.43 (2.47)	ns	slf
Pardini et al. 2009	Whole-brain & ROI	Autism	10	10	19.70 (2.83)	19.90 (2.64)	↓	l-orbitofrontal cortex, anterior cingulate, medial frontal gyrus, inferior frontal gyrus, r-superior frontal gyrus
Langen et al. 2007	tractography	Autism	21	22	25.57 (6.08)	28.45 (6.39)	↓	putamen tract