

# Diffusion Tensor Imaging in Young Children with Autism: Biological Effects and Potential Confounds

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**Background:** Diffusion tensor imaging (DTI) has been used over the past decade to study structural differences in the brains of children with autism compared with typically developing children. These studies generally find reduced fractional anisotropy (FA) and increased mean diffusivity (MD) in children with autism; however, the regional pattern of findings varies greatly.

**Methods:** We used DTI to investigate the brains of sedated children with autism ( $n = 39$ ) and naturally asleep typically developing children ( $n = 39$ ) between 2 and 8 years of age. Tract based spatial statistics and whole brain voxel-wise analysis were performed to investigate the regional distribution of differences between groups.

**Results:** In children with autism, we found significantly reduced FA in widespread regions and increased MD only in posterior brain regions. Significant age  $\times$  group interaction was found, indicating a difference in developmental trends of FA and MD between children with autism and typically developing children. The magnitude of the measured differences between groups was small, on the order of approximately 1%–2%. Subjects and control subjects showed distinct regional differences in imaging artifacts that can affect DTI measures.

**Conclusions:** We found statistically significant differences in DTI metrics between children with autism and typically developing children, including different developmental trends of these metrics. However, this study indicates that between-group differences in DTI studies of autism should be interpreted with caution, because their small magnitude make these measurements particularly vulnerable to the effects of artifacts and confounds, which might lead to false positive and/or false negative biological inferences.

**Key Words:** Autism, diffusion tensor imaging, fractional anisotropy, mean diffusivity, MRI, white matter

Autism is a neurodevelopmental disorder defined by impairments in communication and social interaction, along with repetitive behaviors and restricted interests (1). Although research on autism has expanded in recent years, the neuropathology of the disorder remains poorly understood (2,3). A growing body of evidence suggests that autism is a disorder of neural connectivity (4,5). Functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) studies demonstrate aberrant functional connectivity during rest (6) and during active tasks such as sentence comprehension (7), working memory (8,9), and attribution of mental states to animated shapes (10). Given that aberrant functional connectivity might result from pathology of the white matter axonal connections, the next logical step is to understand the underlying structural architecture of the white matter of the brain.

Diffusion tensor imaging (DTI) (11) is a magnetic resonance imaging (MRI) technique that allows *in vivo* investigation of compositional, microstructural, and architectural characteristics of tissues (12,13) and is currently the most common method for examining the architecture of white matter in the human brain. A number of

DTI studies of autism with normal control comparisons have been published, which include a range of autism spectrum disorders (ASD), including autistic disorder, Asperger syndrome, and pervasive developmental disorder—not otherwise specified (PDD-NOS). Results vary widely across studies—for example, with voxel-wise analysis techniques, researchers found reduced FA in frontal and temporal white matter in children with high-functioning autism (HFA) (14,15), reduced FA within and near the corpus callosum and internal capsule in a group of individuals with HFA that span childhood through adulthood (16), and widespread reductions in FA throughout the white matter in a group of children with ASD (17). With a volume-of-interest approach, reductions in FA were found in the corpus callosum (18) and the superior temporal gyrus and temporal stem (19) of children and young adults with autism and PDD-NOS. Tractography studies found reduced FA in the frontal lobe of children with ASD (20) and the cerebellum of adults with Asperger syndrome (21). With multiple techniques, reduced FA was found in the uncinate fasciculus, arcuate fasciculus, cingulum, and corpus callosum in young children with ASD (22). A few studies have found increased FA in children and adolescents with ASD (23–25). Mean diffusivity (MD) is less frequently reported; however, studies mainly found either no difference or higher MD in subjects with autism compared with typically developing subjects in a variety of brain regions (26–28), although some find reduced MD in autism (29). The subject demographic data, analysis approach, and overall results of these studies are summarized in Table 1.

Although the variability in methods used (voxel-wise, volume-of-interest, and/or tractography) and differences in characteristics of participants (age, sex, and/or diagnosis) across studies limits direct comparison, most works found reduced FA and increased MD in children and adults with ASD, but the regional pattern of findings is heterogeneous. Although some regions were identified as having statistically significant differences of FA across multiple studies, few if any regions were reproduced in all studies. For example, multiple studies identified differences in the internal capsule (17,23,27,30–32), external capsule (16,17,30), superior temporal

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**Table 1.** Summary of Relevant Literature

Reference	Year	Diagnosis	Analysis Method	<i>n</i>		Age Range/Mean (yrs)		Result		Affected Brain Regions
				AUT	TYP	AUT	TYP	FA	MD	
Ben Bashat (23)	2007	Autism	ROI	7	41	1.8–3.3	.25–23	↓, ↑	NR	cst, cc, plic, ec
Weinstein (25)	2011	Autism	TBSS, ROI, Tract	22	32	1.5–5.8	1.5–5.8	↑	NS	cc, slf, cg
Sundaram (20)	2008	ASD	Tract	50	16	4.79	6.84	↓	↑	frontal
Jeong (53)	2011	ASD	Tract	32	14	4.9	5.6	↓	NR	af, uf, cc
Kumar (22)	2010	ASD	TBSS	32	16	2.5–8.9	2.5–8.6	↓	↑	uf, af, cc, cg, ifo
Mengotti (29)	2011	Autism	ROI	20	22	4–14	4–11	NR	↑, ↓	frontal, cc
Peters (28)	2012	TSC	Tract	40	29	.5–25	.9–25	↓	↑	cc
Hong (65)	2011	HFA	Tract	18	16	8.69	9.81	NS	↑	cc
Ke (15)	2009	HFA	SPM	12	10	8.75	9.4	↓	NR	frontal, stg
Barnea-Goraly (30)	2010	ASD	TBSS	13	11	10.5	9.6	↓	NR	widespread WM
Jou (17)	2011	ASD	TBSS	15	8	4.9–17.0	8.9–16.7	↓	NR	widespread WM
Cheon (34)	2011	HFA	TBSS	17	17	8–14	8–14	↓	↑	uf, ilf, cc, thal
Shukla (27)	2010	ASD	ROI	26	24	9–18	9–19	↓	↑	cc, ic, mcp
Shukla (55)	2011	ASD	TBSS	26	24	9–18	9–19	↓	↑	frontal, temporal, parietal
Shukla (32)	2011	ASD	TBSS	26	24	9–20	9–19	↓	↑	widespread WM
Cheng (31)	2010	ASD	TBSS	25	25	10–18	10–18	↓, ↑	NR	widespread WM
Noriuchi (33)	2010	HFA	SPM	7	7	11–18	11–18	↓	NR	cc, cg, slf, dlpc, amyg, temporal
Fletcher (66)	2010	HFA	VOI	10	10	11–17	11–16	NS	↑	af
Groen (26)	2011	HFA	SPM	17	25	12–18	12–18	↓	↑	widespread WM
Barnea-Goraly (14)	2004	HFA	SPM	7	9	14.6	13.4	↓	NR	cc, cg, fontal, temporal, amyg
Bode (24)	2011	HFA	TBSS	27	26	11.4–17.6	11.7–17.3	↑	NS	or, ifo
Alexander (18)	2007	ASD	ROI	43	34	7–33	8–29	↓	↑	cc
Lee (19)	2007	ASD	ROI	43	34	7–33	8–29	↓	↑	stg, ts
Keller (16)	2007	HFA	SPM	34	31	10–35	10–35	↓	NR	cc, ec
Pardini (67)	2009	LFA	SPM, Tract	10	10	18–27	18–26	↓	NR	ofc, uf
Thakkar (68)	2008	ASD	ROI	12	14	30	27	↓	NR	acc
Catani (21)	2008	Asperger	Tract	15	16	18–49	18–49	↓	NS	scp, cerebellum

Summary of relevant literature on diffusion tensor imaging (DTI) and autism (AUT), including results of fractional anisotropy (FA) and mean diffusivity (MD) differences in children with AUT compared with typical development (TYP), and a listing of brain regions where statistically significant differences were found. Please note that some studies looked at the whole brain, whereas others targeted specific regions; thus, reported regions should be interpreted while taking into account the analysis method used.

acc, anterior cingulate cortex; af, arcuate fasciculus; amyg, amygdale; ASD, autism spectrum disorders; cc, corpus callosum; cg, cingulum; cst, cortico-spinal tract; dlpc, dorsolateral prefrontal cortex; ec, external capsule; HFA, high-functioning autism; ic, internal capsule; icp, inferior cerebellar peduncle; ifo, inferior fronto-occipital fasciculus; ilf, inferior longitudinal fasciculus; LFA, low-functioning autism; mcp, middle cerebellar peduncle; NR, not reported; NS, reported as nonsignificant; ofc, orbitofrontal cortex; or, optic radiation; plic, posterior limb of the internal capsule; ROI, region of interest; scp, superior cerebellar peduncle; slf, superior longitudinal fasciculus; SPM, statistical parametric mapping; stg, superior temporal gyrus; TBSS, tract based spatial statistics; thal, thalamic radiation; Tract, tractography; ts, temporal stem; TSC, tuberous sclerosis complex; uf, uncinate fasciculus; VOI, volume of interest; WM, white matter; ↓, reduced in AUT compared with TYP; ↑, increased in AUT compared with TYP.

**Table 2.** Subject Demographic Data

Characteristic	TYP ( <i>n</i> = 39)	AUT ( <i>n</i> = 39)	<i>p</i>
Male/Female	26/13	28/11	.624
Age (Years), Mean $\pm$ SD	4.74 $\pm$ 1.76	4.63 $\pm$ 1.76	.783
Age Range (Years)	2.02–8.09	2.19–8.72	
Handedness, Left/Right/Both/n/a	2/28/5/4	4/16/8/11	
Race			
White	31	28	
Black or African American	3	7	
Asian	2	2	
Multiple races	3	1	
Unknown	0	1	
Behavioral Measures (Mean $\pm$ SD)			
Nonverbal DQ	113.11 $\pm$ 16.07	60.97 $\pm$ 18.90	<.000001 <sup>a</sup>
Nonverbal DQ Range	88.82–150.00	32.79–109.18	
ADOS Social Affect	.86 $\pm$ 1.03	14.22 $\pm$ 2.97	<.000001 <sup>a</sup>
ADOS Restricted/Repetitive Behavior	.53 $\pm$ .77	5.49 $\pm$ 1.79	<.000001 <sup>a</sup>

Demographic data of the subject groups, including age, gender, handedness, race, and results of behavioral tests. ADOS, Autism Diagnostic Observation Schedule; AUT, autism; DQ, developmental quotient; n/a, handedness not established; TYP, typically developing.

<sup>a</sup>Significant difference between groups.

gyrus (15,17,19,30,31), amygdala (14,30,33), and thalamus/thalamic radiation (17,32,34), without consensus. There is also no apparent pattern of regional differences on the basis of age. For example, the corpus callosum was found to have a statistically significant difference of FA in studies spanning all ages and across various ASD diagnoses. Yet others did not find it to be different (15,24,26,31). Furthermore, although regional patterns might exist on the basis of other characteristics such as severity of symptoms, cognitive skills, and at younger ages, there are insufficient studies examining these factors to make meaningful inferences.

In this work we used DTI to investigate differences between brains of a large sample of children 2–8 years of age who met full diagnostic criteria for autism (AUT) and an age- and gender-matched sample of typically developing children (TYP). Voxel-wise analyses were performed with typical methods (35) for comparison with literature data, along with comparative analysis of average brain white matter morphology between groups (36). The goal of this work is to characterize the regional distribution of differences in the brains of children with autism compared with TYP in an age range that is underreported in the literature. We also comment on the influence of confounds in DTI data and their potential impact on results of clinical DTI analyses.

## Methods and Materials

Subject groups are composed of 39 AUT who met DSM-IV criteria for autism (1) and 39 TYP. Demographic and diagnostic information are provided in Table 2. Parents of all participating subjects provided written informed consent for study participation, which was approved by the National Institutes of Health Combined Neurosciences Institutional Review Board.

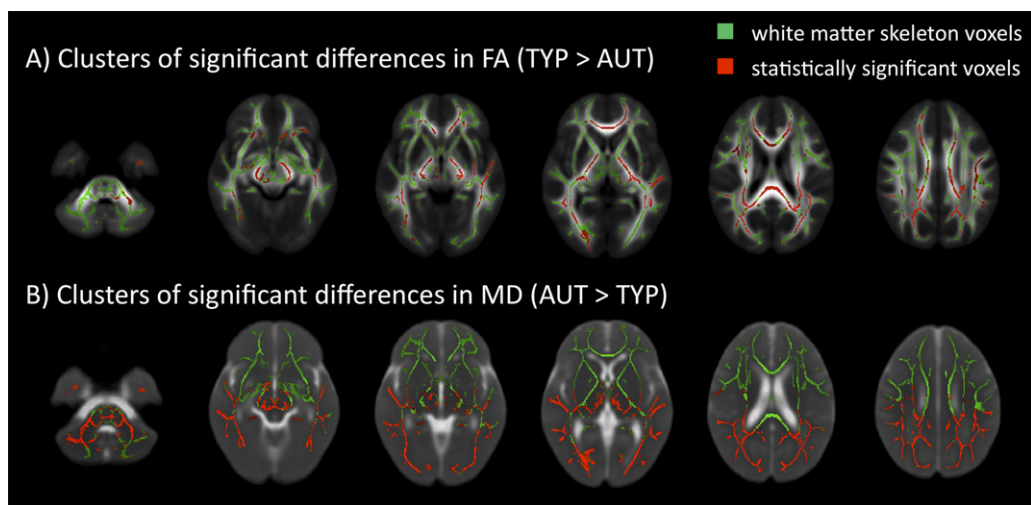
Children with autism (AUT) were included after an evaluation consisting of research-reliable administrations of the Autism Diagnostic Interview-Revised (37), the Autism Diagnostic Observation Schedule (38), and clinical judgment of a best estimate diagnosis by qualified doctoral level clinicians at the National Institutes of Health. Typically developing children (TYP) were assessed with cognitive measures, administration of the Autism Diagnostic Observation Schedule, and parent-report questionnaires such as the Social Communication Questionnaire (39) and the Child Behavior Check-

list (40). The TYP exclusion criteria included the presence of any cognitive impairments or signs of an autism spectrum disorder or a history of a substantial medical, neurological, or developmental disorder. The nonverbal developmental quotients of children were estimated on the basis of age-appropriate and developmentally appropriate cognitive/developmental tests (Mullen Scales of Early Learning (41) or Differential Ability Scales, Second Edition (42)).

Children with autism were scanned under sedation with propofol, and TYP were scanned during natural sleep, without sedation, on a 1.5T GE scanner (General Electric, Milwaukee, Wisconsin) with an eight-channel head coil. The DTI data were acquired with single shot, spin-echo, echo-planar imaging with 2.5-mm isotropic voxels (echo/repetition time approximately 84.5/21,330 msec). The DTI datasets consisted of 80 imaging volumes: 50 independent gradient directions at  $b = 1100 \text{ sec/mm}^2$ , 10 directions at  $b = 300 \text{ sec/mm}^2$ , and 10 non-diffusion weighted images ( $b = 0 \text{ sec/mm}^2$ ). At the beginning of the study a longer protocol was used with a larger number of intermediate  $b$ -values. Both protocols were found to be equivalent in quality. Diffusion weighted images went through a comprehensive correction pipeline with TORTOISE (43), which registers the volumes of a DTI dataset to reduce the effects of motion, eddy current distortions (44), and echo-planar imaging distortions (45). Corrections were performed in the native space of each subject, and appropriate rotations were applied to the  $b$ -matrix (44,46). More details about the scanning protocol and correction pipeline are provided in Supplement 1.

After corrections, robust estimation of tensors by outlier rejection (RESTORE) (47) was used to estimate the diffusion tensor and tensor derived metrics. The RESTORE algorithm was selected for its ability to detect and remove artifactual data points on a voxel-wise basis, correcting for subtle artifacts such as cardiac pulsation and respiration signal drop-outs, which has been shown to be an important consideration in clinical population analyses of DTI data (48). In this study we focus on analyses of FA and MD and also report results of axial (AD) and radial diffusivities (RD).

Voxel-wise analysis was performed with tract based spatial statistics (TBSS) (35), with statistics computed only in the voxels of the white matter skeleton ( $FA > .2$ ). Images were registered to the FMRIB58\_FA standard space template as recommended for the



**Figure 1.** Results of tract based spatial statistics analysis of **(A)** fractional anisotropy (FA) and **(B)** mean diffusivity (MD) overlaid on mean FA and MD computed from all subjects. Differences between autism (AUT) and typical development (TYP) were observed in widespread regions of FA and large clusters predominantly in posterior regions of MD. Statistically significant voxels are displayed in red; white matter skeleton voxels are displayed in green. Significance threshold was set at  $p < .05$  corrected for multiple comparisons with family wise error and controlled for age.

TBSS pipeline. Permutation-based nonparametric statistics (Randomise [49], FSL [50]) were used with 10,000 permutations, controlling for age by using it as a covariate in the model, with threshold free cluster enhancement and corrected for multiple comparisons with family wise error correction ( $p < .05$ ).

The TBSS analysis is limited, by design, to only the most central portion of the white matter tracts (the white matter skeleton). To assess the whole brain, tensor-based registration was used to register the diffusion tensors from all children, AUT and TYP, into a common space with DTI-TK (36,51). This type of registration uses the directional information of the diffusion tensor to align the data of all subjects with a high level of agreement. Randomise (49) was used on the DTI-TK registered images to perform voxel-wise statistics in the whole brain, with threshold free cluster enhancement optimized for three-dimensional data.

In addition to whole brain voxel-wise analysis, population average tensors were created from the DTI-TK registered tensors for each group. Subtraction maps were then computed for average FA and MD to assess the magnitude of the between-group differences both visually and quantitatively, with regions of interest. See Supplement 1 for details on region of interest placement.

## Results

### TBSS Analysis

The TBSS analysis found lower FA in AUT compared with TYP throughout the white matter, including but not limited to the cerebellum, genu of the corpus callosum, splenium of the corpus callosum, body of the corpus callosum, corticospinal tract, pons, posterior limbs of internal capsule, left superior temporal gyrus, superior-anterior, and temporal-parietal regions (Figure 1A). MD was greater in AUT compared with TYP in most white matter regions but only in the posterior half of the brain (Figure 1B). Both AD and RD were greater in AUT compared with TYP, with a pattern that closely matches the MD findings.

In addition to group differences, an age  $\times$  group interaction was found throughout the white matter. This includes nearly all (>99%) of voxels found to have statistically significant group difference of FA and MD and many more (Figure 2). The age interaction indicates that FA increases with age in both groups but increases faster in TYP

than in AUT, whereas MD and RD decrease with age in both groups but decrease faster in AUT than in TYP. The AD results also closely matched the MD results with the exception of superior-anterior regions, where AD decreases faster in TYP. Note again the fewer significant voxels observed in the superior-anterior portions of the brain. Average developmental trends of FA and MD in all statistically significantly different voxels are shown in Figure 2, and selected regional plots are provided in Supplemental Figures S1 and S2 in Supplement 1.

### Whole Brain Statistical Analysis

Statistical analysis of DTI-TK registered data revealed similar findings of reduced FA and increased MD in AUT but with some marked differences, compared with the TBSS results. Regions with reduced FA were in similar locations as those from the TBSS analysis but largely failed to reach statistical significance. In addition, FA was found to be greater in AUT than TYP in a small cluster in the cerebellum. Also similar to TBSS results, MD was greater in AUT than TYP with large clusters in inferior-posterior areas of the brain but none in the anterior-superior areas. These clusters extend beyond the white matter to include large portions of cortical gray matter and thalamus. In a small superior-anterior cortical area MD was greater in TYP than AUT (Supplemental Figure S3 in Supplement 1).

Age  $\times$  group interaction was observed in large portions of the brain parenchyma (Figure 3). Fractional anisotropy increases faster in TYP than AUT in many white matter structures and also in the basal ganglia. In the cerebellum FA increases faster in TYP, but the trajectory is relatively flat with age in AUT. For MD, the age interaction was more of a global effect, including much of the sub-cortical white matter and cortical gray matter in the posterior portions of the brain, with MD decreasing faster in AUT than TYP. Again, a difference between superior-anterior and inferior-posterior portions of the brain was present.

### Whole Brain Average Morphology

Average directionally encoded color (52), FA, and MD maps from the AUT and TYP groups are strikingly similar when viewed qualitatively (Supplemental Figure S4 in Supplement 1). Subtraction of average FA and MD (AUT minus TYP) shows very small magnitude differences between groups (Supplemental Figure S5 in Supple-



**Figure 2.** Age  $\times$  group interaction for (A) FA and (B) MD from tract based spatial statistics analysis, overlaid on mean FA and MD computed from all subjects. The FA increases with age in both groups but increases faster in TYP than in AUT, whereas MD decreases with age in both groups but decreases faster in AUT than in TYP. Trends are demonstrated in plots of the average of all significant voxels of FA and MD versus age, respectively. Abbreviations as in Figure 1.

ment 1). Region of interest analysis revealed differences of average FA and MD of approximately 1%–2% between AUT and TYP (Supplemental Table S1 in Supplement 1).

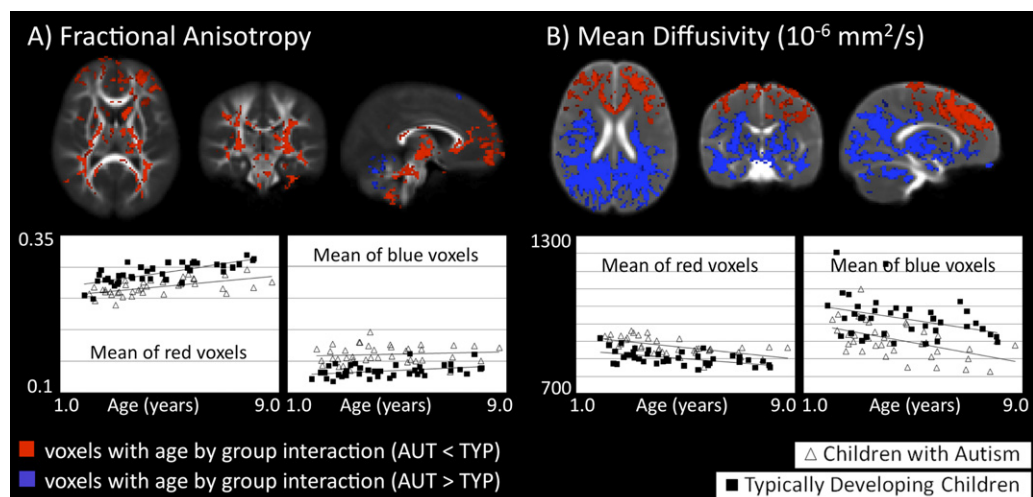
#### Impact of Artifacts

The curious pattern of MD differences between superior-anterior and inferior-posterior regions suggest that an artifact or confound without anatomical origin might be impacting the MD findings of this study. This was hypothesized because of the abrupt border between statistically significant and nonsignificant voxels (Figure 1), which does not coincide with anatomical structures. One of the outputs of RESTORE is a map of the artifactual voxels detected in the diffusion weighted images of each dataset. A pattern of artifacts not conforming to the expected distribution (48) was detected in six subjects: five TYP, and one AUT. This pattern includes

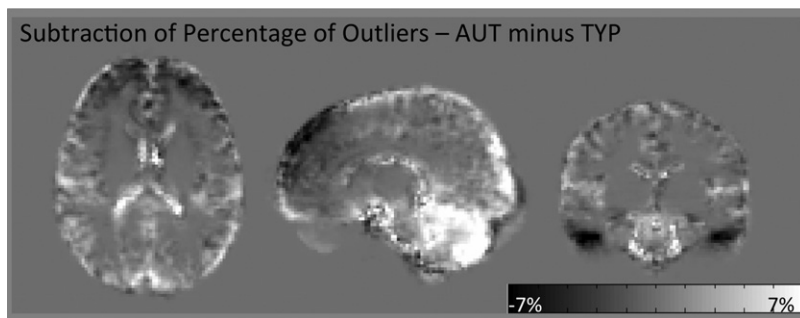
a large number of artifactual voxels in superior-anterior regions of the brain (Supplemental Figure S7 in Supplement 1). We computed the average distribution of artifactual voxels for AUT and TYP groups and present the subtraction of these maps (AUT minus TYP) in Figure 4. The subtraction shows a different pattern of artifacts in each population, with a greater overall incidence of artifacts in the AUT group, particularly in the cerebellum, and in the TYP group a greater incidence of artifacts in superior-anterior and temporal regions.

#### Discussion

Investigation of DTI data revealed widespread differences of FA and MD in brain parenchyma of a group of young children who met



**Figure 3.** Age  $\times$  group interaction for (A) FA and (B) MD from whole brain analysis, overlaid on mean FA and MD computed from all subjects. Red voxels indicate regions where metrics are greater in TYP than AUT, and blue voxels indicate regions where metrics are greater in AUT than TYP. Plots for red and blue voxels are presented to demonstrate average trends with age. Abbreviations as in Figure 1.



**Figure 4.** Subtraction of maps of the average distribution of artifactual data points (AUT minus TYP). Bright voxels indicate a higher incidence of artifacts in AUT, dark voxels indicate a higher incidence of artifacts in TYP, and gray (background) voxels are exactly equal to zero. Note the distinctly different distribution of artifacts between groups, with greater incidence in the cerebellum for AUT and greater incidence in superior-anterior and temporal regions in TYP. Abbreviations as in Figure 1.

full diagnostic criteria for autism (AUT), compared with TYP, with a predominant pattern of reduced FA and increased MD in AUT.

Only two other DTI studies of ASD coincide closely with our age range (22,53). These studies similarly find reduced FA and increased MD in AUT compared with TYP. Regionally, our study is generally consistent with these studies but also implicates more widespread regions. At our age range and older, most studies have also reported reduced FA and increased MD in ASD; however, the regions affected are inconsistent across studies. For instance, some report findings in isolated regions (26), whereas others report widespread differences (27,30). This discrepancy in regional pattern has led different studies to propose various interpretations of physiology and behavior. Differences in the frontal lobes were attributed to functional deficits in social cognition (15,22), differences in temporal regions are linked to socio-emotional and language deficits (17,22), and differences in the corpus callosum are purported to reflect nonverbal cognition (18) or repetitive behavior (54), among other interpretations. The widespread regional distribution of differences found in this work favor an interpretative hypothesis of global differences, similar to the findings of Shukla *et al.* (27,32,55), with no specificity to any one brain “network.”

In addition to group differences in mean value of tensor derived metrics, we found significant age  $\times$  group interactions in the brain parenchyma, with anisotropy increasing with age and diffusivity decreasing with age but at different rates for the two groups. Moreover, as demonstrated in Supplemental Figures S1–S2 in Supplement 1, the trends are regionally dependent. For example, trends in the genu of the corpus callosum and splenium of the corpus callosum are fairly flat over our age range, in contrast to regions such as the posterior limbs of internal capsule. This is in agreement with previous studies in healthy children (56,57), where FA and MD values in the corpus callosum generally plateau earlier in development and might not be well-modeled by linear regression in our age range. Interestingly, studies in very young subjects have shown the opposite trend—mainly increased FA and nonsignificant MD findings in ASD (23,25). The different trends with age between groups observed in this work suggest that the difference of FA between AUT and TYP is less pronounced at younger ages and increases with age. One could hypothesize that the developmental trends of FA might cross at ages younger than were included in this study, resulting in a reversal of the trends of FA at an earlier developmental stage. In support of this hypothesis, a recent longitudinal DTI study of autism showed differential developmental trajectories of FA, with an average cross-over of the trajectories occurring between 6 and 16 months of age, depending on the region (58). In addition to DTI findings, atypical brain volume growth patterns have been previously reported in autism (59), with a specific indication of early over-growth in the first year of life (60). Given the crucial role that neurodevelopment plays in the pathophysiology and clinical presentation of autism, abnormalities in developmental trajectories

are potentially more interesting and informative than the absolute differences of tensor derived metrics and deserve further study, including longitudinal investigations of large groups of children with autism and children with typical development followed from early childhood through the key developmental ages.

Although significant group differences in DTI metrics were found, it is important to note that the difference in measured FA and MD values between groups is modest, on the order of approximately 1%–2%. A close review of studies that include regional mean and SD values also suggest that group differences are small (17,19,22,25). We notice substantial overlap in the distribution of the values of DTI metrics between groups. Although not explicitly mentioned in previous works that present scatter plots of diffusion metrics (16,28,32), overlap can also be noticed in their data. Examination of the overlap and spread of data in the scatter plots suggests that many of the AUT subjects cannot be distinguished from TYP subjects on the basis of the value of DTI metrics alone. However, a small subset of AUT subjects are further removed from the TYP subjects, thereby driving the statistically significant differences detected in this work. This fact suggests a potential biological heterogeneity of the brains of children with autism, even within a diagnostically homogenous group.

The small magnitude of between-group differences has potential repercussions on the analysis and interpretation of DTI data. The primary concern is that experimental artifacts or other confounding factors might affect the AUT and TYP groups differently, such that an experimental confound of large magnitude might overshadow smaller differences of biological origin or even create false positive findings. We found a spatial distribution of statistical significance in MD that suggests the presence of this phenomenon in our data. Specifically, MD was significantly greater in AUT than TYP in the inferior-posterior regions of the brain, was not significantly different in a middle band, and finally was significantly greater in TYP than AUT in the superior-anterior region of the brain (Figure 3). We believe, on the basis of consistency with previous literature, that the finding in the posterior region has biological origin but that artifacts have destroyed significance in the middle regions of the brain and produced false positive results in the superior-anterior region. In fact, motion artifacts present in the diffusion weighted imaging data were more pronounced specifically in the superior-anterior regions in TYP subjects.

The different pattern of artifacts for the two groups could be attributed to the fact that AUT were scanned under sedation, whereas TYP were scanned during natural sleep. The un-sedated TYP were more likely to move during the scan than the sedated children with autism. This might be consistent with the observed greater incidence of artifacts in superior-anterior regions for TYP, given the recent fMRI results showing a significant confound of motion on resting state fMRI results primarily in the frontal lobes (61).

Additionally, sedation might result in altered patterns of physiological noise (i.e., a systematically different heart rate and breathing pattern), compared with non-sedated subjects. These types of artifacts are known to have a regionally varying effect on DTI data, with predominant effects in the cerebellum (48,62). Although RESTORE tensor fitting used in this study is intended to correct for artifacts such as physiological noise on a voxel-wise basis, the algorithm might break down in cases where the number of artifactual voxels is greater than the number of good ones (47). Thus, this analysis contains an example of correctable artifacts (i.e., cerebellum) and uncorrectable artifacts (i.e., superior-anterior).

Although DTI is a promising method for revealing anatomic abnormalities in the brains of subjects with autism, caution must be exercised in interpreting between-group differences. Because of the consistent nature of findings of reduced FA and increased MD in the literature and replicated in our work, the results of this study are likely related to between-group differences with a biological origin, perhaps related to a fundamental difference in underlying brain structure between autism and typical development. Due to the small magnitude of these differences, however, these particular measures are unlikely to be useful biomarkers for single subject diagnoses. Furthermore, the lack of reproducible regional findings across studies implies that specifics of analysis method, group size, and sample heterogeneity are likely to play a role in the particular regions identified by a study. Indeed, in this work, the use of two slightly different approaches to data analysis (TBSS and whole brain voxel-wise analysis) resulted in very different levels of statistically significant differences in FA; the pattern of between-group differences remained the same, but with TBSS the results reached significance thresholds, whereas only a few voxels reached this same threshold with whole brain analysis. This difference might be due simply to strict penalties incurred for increased multiple comparisons. If differences between these groups are truly widespread, hypothesis-driven studies that focus on searching for differences in a few specific regions might not be the correct approach. In fact, these studies, which are intrinsically more powerful than whole brain voxel-wise analyses, might lead to the false conclusion that the specific region(s) tested is uniquely implicated in autism.

Finally, one might question the histopathological underpinnings of the FA and MD differences observed between AUT and TYP. It is tempting to attribute these differences to a specific biological feature of the brain, but unfortunately, without independent histological validation this type of inference is rarely warranted from DTI data. In the literature, FA differences are frequently reported as an indication of “white matter integrity.” Although white matter disruption might result in reduced diffusion anisotropy, equating FA to a specific measure of integrity of white matter is misleading. For example, in healthy white matter the underlying organization of fibers heavily influences the value of anisotropy (i.e., the more coherent the fiber organization, the higher the measured value of FA [12]. In regions with complex white matter architecture, FA might even paradoxically increase after white matter degeneration (63). More recently, it was shown that FA values increased in perilesioned cortex after traumatic brain injury in a rat model, because of organized gliosis, not axonal regeneration (64). In healthy white matter, diffusion anisotropy is known to increase during post-natal maturation (53), although the relative importance of each specific maturational process (i.e., change in the size of the extracellular space, composition of the extracellular matrix, and degree of myelination) in driving the FA increase is not clear. MD generally decreases during post-natal maturation. By contrast, degenerative processes also often result in reduced FA and increased MD. Our finding of reduced FA and increased MD in AUT cannot be attrib-

uted to a single biological factor, given these considerations. Because the FA and MD changes are widespread, the abnormality does not seem to involve any one specific brain structure. However, this pattern could be consistent with global differences in the level of tissue maturity between groups, abnormal maturation, or degenerative processes.

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