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Alterations in Frontal Lobe Tracts and Corpus Callosum in Young Children with Autism Spectrum Disorder

Ajay Kumar[1,2], Senthil K. Sundaram[1,2], Lalitha Sivaswamy[1,2], Michael E. Behen[1,2], Malek I. Makki[2,3], Joel Ager[4], James Janisse[4], Harry T. Chugani[1,2] and Diane C. Chugani[1,3,5]

[1]Carman and Ann Adams Department of Pediatrics, and the [2]Departments of Neurology and [3]Radiology, and [4]Family Medicine & Community Health, Children's Hospital of Michigan, Detroit Medical Center, Wayne State University, School of Medicine, Detroit, Michigan, USA and [5]Department of Pharmacology, Children's Hospital of Michigan, Detroit Medical Center, Wayne State University, School of Medicine, Detroit, Michigan, USA

Major frontal lobe tracts and corpus callosum (CC) were investigated in 32 children with autism spectrum disorder (ASD, mean age: 5 years), 12 nonautistic developmentally impaired children (DI, mean age: 4.6 years), and 16 typically developing children (TD, mean age: 5.5 years) using diffusion tensor imaging tractography and tract-based spatial statistics. Various diffusion and geometric properties were calculated for uncinate fasciculus (UF), inferior fronto-occipital fasciculus (IFO), arcuate fasciculus (AF), cingulum (Cg), CC, and corticospinal tract. Fractional anisotropy was lower in the right UF, right Cg and CC in ASD and DI children; in right AF in ASD children; and in bilateral IFO in DI children, compared with TD children. Apparent diffusion coefficient was increased in right AF in both ASD and DI children. The ASD group showed shorter length of left UF and increased length, volume, and density of right UF; increased length and density of CC; and higher density of left Cg, compared with the TD group. Compared with DI group, ASD group had increased length, volume, and density of right UF; higher volume of left UF; and increased length of right AF and CC. Volume of bilateral UF and right AF and fiber density of left UF were positively associated with autistic features.

Keywords: association fiber tracts, autism spectrum disorder, children, corpus callosum, corticospinal tract, developmental impairment, diffusion tensor imaging, frontal lobe

Introduction

Autism spectrum disorders (ASDs) are neurodevelopmental behaviorally defined conditions characterized by impaired language and reciprocal social interaction, as well as presence of repetitive and stereotypical behaviors. Abnormalities within specific frontal lobe regions may play an important role in the manifestation of the core features of autism. For example, there is functional MRI (fMRI) evidence to suggest that the early social deficits of autism may be related to a social cognition network in which frontal lobe plays a crucial role (Baron-Cohen et al. 1999). A number of structural and functional neuroimaging studies have found abnormalities in the frontal lobe of patients with autism; these abnormalities appear to correlate with deficits of social cognition, executive function, communication, and repetitive behavior (Carper and Courchesne 2000, 2005; Chandana et al. 2005; Chugani et al. 1999; Ohnishi et al. 2000; Luna et al. 2002; Herbert et al. 2003, 2004; Salmond et al. 2003; Hazlett et al. 2004). Previous studies using positron emission tomography from our group had found serotonergic functional abnormalities in the frontal lobe of autistic children (Chugani et al. 1999; Chandana et al. 2005). Specifically, decreased serotonin synthesis capacity in the frontal cortex and an abnormal developmental trajectory for whole-brain serotonin synthesis were observed (Chugani et al. 1998, 1999). Since serotonin acts as a neurotrophic factor early in life and modulates axonal arborization (Vitalis and Parnavelas 2003), we hypothesized that the observed developmental alterations in serotonin synthesis may be related to abnormalities in underlying connectivity of frontal lobe.

Brain connectivity in autism has been investigated using a number of techniques, including electroencephalography, fMRI, and diffusion tensor imaging (DTI). Though functional connectivity can be demonstrated by electroencephalography and fMRI, these techniques do not directly assess the structural integrity of the neural networks. DTI remains the only non-invasive method, which can directly evaluate structural connectivity in the brain. However, to date there are only few studies reporting DTI abnormalities in autism (Barnea-Goraly et al. 2004; Alexander et al. 2007; Ben Bashat et al. 2007; Keller et al. 2007; Lee et al. 2007, 2009; Catani et al. 2008; Sundaram et al. 2008b; Thakkar et al. 2008). In a recent DTI study of frontal lobe fibers from our group (Sundaram et al. 2008b), abnormalities in the diffusion (anisotropy and diffusivity) and geometric (fiber length and fiber volume) properties of long and short association fibers of frontal lobe were observed. The distribution of fiber length was found to differ between the control and ASD groups. In particular, the fiber length distribution was significantly more negatively skewed in the ASD group compared with controls. Histograms of fiber lengths showed a bimodal distribution with a smaller peak corresponding to long fibers. When the fibers corresponding to this second peak were isolated, the average length of these long fibers was significantly higher in ASD than in control subjects. However, that study did not isolate individual association tracts that could explain the anatomic location of the fiber changes within the frontal lobe. Hence, the current study was designed to investigate the major association tracts of the frontal lobe including the uncinate fasciculus (UF), inferior fronto-occipital fasciculus (IFO), arcuate fasciculus (AF), and the cingulum (Cg), as well as the corpus callosum (CC) in young children with ASD compared with typically developing children. In order to evaluate the specificity of the findings, that is, findings specifically related to autistic features, we also investigated another group of children having developmental impairment, but no autistic features (DI). We also investigated the corticospinal tract (CST) as a control tract, which we hypothesized would not be affected in children with ASD or DI.

Methods and Materials

Subjects

Thirty-two children with diagnoses of ASD (ASD group, mean age: 5.0 years; range: 2.5–8.9 years, 29 males and 3 females), 12 nonautistic developmentally impaired children (DI group, mean age: 4.6 years; range: 3.0–9.0 years, 10 males and 2 females), and 16 typically developing

children (TD group, mean age: 5.5 years; range: 2.5–8.6 years, 12 males and 4 females) underwent MRI with DTI and developmental and behavioral assessments. All the patients were referred by the Children's Hospital of Michigan Neurology Clinic, and some of these children were reported previously (Sundaram et al. 2008b).

Inclusion criteria for the "ASD group" included the following: 1) a diagnosis of Autistic Disorder, Asperger's Disorder, or Pervasive Developmental Disorder not-otherwise-specified made by pediatric neurologists using DSM-IV TR criteria and 2) scores above the cutoff on the Social Communication Questionnaire (SCQ, Rutter et al. 2003) and Autism Quotient (AQ) ≥ 85 on the Autism Quotient of the Gilliam Autism Rating Scales (GARS) (Gilliam 1995). We did not attempt to distinguish between different diagnostic categories within the ASD group, as current data does not support the notion that strictly behaviorally defined autism is a homogeneous disorder. Conversely, persons on the spectrum in different categories may have the underlying etiology (Bill and Geschwind 2009). Therefore, the aim of the present study was to investigate the major association tracts in young children with ASD compared with typically developing children and children with developmental impairment without autistic features.

The inclusion criterion for the "DI group" included the following: 1) measured functioning in at least one domain of adaptive behavior functioning <70; 2) SCQ total score below the cutoff for ASD; and 3) AQ < 80.

Inclusion criteria for the typically developing (TD) group included the following: 1) measured intellectual functioning within normal limits ($\geq$85); 2) normal neurological screening; and 3) absence of any current or historical medical or psychiatric diagnoses. TD children were obtained through active recruitment, and a compensation of $100 was offered to all TD subjects for participation in the study.

Children with any of the following were excluded from the study: 1) history of seizures; 2) focal deficits on clinical examination by a pediatric neurologist; 3) MRI interpreted as abnormal by a pediatric neuroradiologist; 4) dysmorphic features suggestive of a genetic syndrome; 5) history of prematurity or perinatal hypoxic-ischemic event; and 6) an inborn error of metabolism, as autistic features can be a part of certain metabolic disorder. The patients were routinely screened for urea cycle disorders and disorders of amino/organic acids by testing for serum ammonia levels, serum amino acid, and urine organic acids, respectively. When the clinical features were suggestive of Smith-Lemli-Opitz syndrome (aggression and screaming spells most specifically), it was ruled out by testing 7-dehydrocholesterol-delta 7-reductase (DHCR7).

All children in the study were right-handed. The groups did not differ on age ($P = 0.24$) or gender ($P = 0.35$). Because the scans for children with ASD or DI were clinical MRI studies, sedation was used as necessary by the sedation team at Children's Hospital of Michigan. None of the control children were sedated for the MRI. However, younger children were scanned while sleeping, and all children were monitored for movement during scan. If there was significant movement, MRI was repeated or those scans were removed from the study. All study participants were studied according to the guidelines of the Human Investigations Committee of Wayne State University. Written, informed consent was obtained from one of the parents or legal guardians of the participants. The Human Investigations Committee at Wayne State University granted permission for the retrieval and analysis of the data that have been obtained clinically for these children.

Neurobehavioral Evaluation

The results of the neuropsychological evaluation are given in Table 1. The developmental and behavioral evaluation included assessment of adaptive behavior functioning, screening for pervasive developmental disorders, and quantification of autism triad symptoms. The Vineland Adaptive Behavior Scales-2nd Edition (VABS, Sparrow et al. 1984) is a caregiver-reported semistructured interview that yields measures of the child's adaptive behavior functioning in 4 domains (communication, daily living, socialization, and motor skills), as well as an overall adaptive behavior composite. The measure is used extensively in research studies on children with developmental disabilities and has excellent reliability and validity (Perry and Factor 1989). The measure was used in the present study to quantify adaptive behavior functioning across all 4 domains in both the ASD and DI groups.

Table 1
Neuropsychological results in ASD and DI children

	ASD group	DI group
VABS adaptive behavior composite	72.8 ± 13.2	76 ± 10.6
VABS communication	71.4 ± 13.7	72.6 ± 8.7
VABS daily living skills	79.8 ± 11.9	83 ± 12.9
VABS socialization	78.7 ± 14.7	84 ± 13.4
VABS motor	77.7 ± 11.6	77.3 ± 12.1
GARS AQ	98.4 ± 11.8	64.8 ± 7.1
GARS Stereotypic Behaviors	9.9 ± 2.2	6.2 ± 1.4
GARS social isolation	9.7 ± 3.4	4.8 ± 1.6
GARS communicative disturbances	9.9 ± 1.6	7.1 ± 1.1

Note: ASD, autism spectrum disorder group; DI, nonautistic developmentally impaired group. VABS is a standard score with a mean of 100 and SD of 15. GARS is a normalized standard score with a mean of 100 and SD of 15 for the autistic population. Each of the subdomains of GARS has a mean of 10 and SD of 3. Values are given as mean ± SD (range).

The SCQ is a caregiver-report measure based on the most sensitive items of the Autism Diagnostic Interview-Revised (Lord et al. 1994) that is widely used as a screening instrument for ASD. The psychometric properties of the scale have been demonstrated to be good, particularly supporting the SCQ as a useful instrument for discriminating ASD from non–ASD problems (Berument et al. 1999). The SCQ was used in the present study to verify the presence (ASD group) or absence of ASD (DI children). For the present study, a cutoff of 15 was used to classify children into the ASD group (Berument et al. 1999; Chandler et al. 2007).

The Gilliam Autism Rating Scale-2nd Edition (Gilliam 1995) is a 42-item behavioral checklist that allows the quantification of frequency and severity of the triad symptoms of autism. The GARS comprises three subscales—stereotyped behaviors, communication, social interaction, and an AQ. The AQ is a standardized score that represents the overall assessment of autistic symptoms displayed by an individual; scores greater than 85 are considered to indicate a "Very Likely" probability of autism diagnosis and indicate the presence of substantial autistic spectrum symptoms. The psychometric properties of the scale have been shown to be good, and the GARS is widely used in clinical and research studies with ASD populations.

For psychometric confirmation of autism diagnosis (and to rule out ASD in children in the DI group), the SCQ was used to ascertain whether the child was above or below the screening cutoff for an autism spectrum disorder and the GARS to verify the overall magnitude of autism triad symptoms present.

Children in the TD group underwent intellectual testing with the age-appropriate Wechsler measures. Children under the age 6 years completed the Wechsler Preschool and Pimary Scale of Intelligence-Third Edition, and children older than 6 years completed the Wechsler Intelligence Scales for Children-Fourth Edition. Both measures are widely used in both clinical and research samples, and the psychometric properties are very good (Sattler 2008).

DTI Acquisition Protocol

MRI scans were performed using a GE system with 3-Tesla magnet (Signa GE Healthcare, Milwaukee, WI). Diffusion tensor images were acquired in the axial plane with diffusion sensitization gradients applied in 6 noncollinear directions and with b value of 1000 s/mm^2. The same imaging parameters were used to acquire T_2-weighted ($b \sim 0$ s/mm^2) images to use as a reference image and to measure the signal attenuation. All image volumes were acquired using 6 averages to increase the signal-to-noise ratio and to reduce image artifacts. The echo time was 79 ms, and the repetition time was about 10 s. A set of minimum 34 axial slices of 3 mm thickness without gap was acquired with matrix size 128*128 and reconstructed to 256*256 matrix covering the whole brain, including the cerebellum. Field of view was 240*240 mm^2, and the approximate scanning time for the DTI acquisition was 9 min. Double refocusing pulse was used to reduce eddy current artifacts, and array spatial sensitivity encoding technique was performed to further reduce the geometric distortion due to the sequence design. As no major artifacts were observed, even at the level of the deep brain structures, no offline correction was done.

Tractography Approach

Acquired diffusion-sensitized and reference image sets were transferred to an Intel Pentium, Microsoft windows-based operating system for further data analysis. Tensor calculation and tractography were performed using DTI studio software version 2.40 (www.mristudio.org). Tractography was carried out based on Fiber Assignment by Continuous Tracking algorithm (Mori et al. 1999). The fiber propagation was stopped at an FA threshold less than 0.2 or an angle threshold greater than 60 degrees.

A brute force fiber tracking was initially performed for the whole brain. Then, individual tracts were isolated by protocols similar to the knowledge-based multiple region approach described for the association tracts previously (Mori et al. 2002). The protocols are described in detail below. The DTI studio software allows isolation of tracts passing through a single region of interest (ROI, using inclusive "OR" operator) or multiple ROIs (using exclusive "AND" operator).

Isolation of Individual Tracts

Uncinate Fasciculus

A 2-ROI approach was used to isolate the UF. The first "OR" ROI was placed in the frontal lobe at the coronal level corresponding to the posterior tip of the caudate, and a second "AND" ROI was placed in the anterior temporal lobe at the same coronal level as the first ROI.

Inferior Fronto-Occipital Fasciculus

Two ROIs were used to isolate the IFO. The first "OR" ROI was placed in the occipital lobe in a coronal slice just posterior to the parieto-occipital sulcus. The second "AND" ROI was placed in the frontal lobe at the location where the frontal and temporal lobes are separated.

Arcuate Fasciculus

For the AF, an ROI was placed in the coronal plane at the level of posterior tip of putamen using the "OR" operator lateral to the superior aspect of the corona radiata. A second ROI in the axial plane was then placed using the "AND" operator at the level just below the Sylvian fissure where the AF can be discretely identified.

Cingulum

The DTI color map was used to isolate Cg as it can be discretely identified in the color map, as a green color bundle just above the CC. Multiple sagittal ROIs around the visible green color were used to extract the Cg.

Corpus Callosum

The CC is the most prominent and easily identifiable tract on standard DTI color maps (shown in red color because of its side-to-side orientation). Three ROIs surrounding the CC were drawn in three consecutive midline sagittal sections to isolate the CC. Because of lack of identifiable boundary between frontal and nonfrontal fibers of CC, no attempt was made to isolate the frontal fibers of the CC.

Corticospinal Tract

The CST was isolated by drawing one ROI around the posterior limb of the internal capsule and another ROI around the CST in the brain stem (identified as a blue color bundle in the anterior part of the brain stem) on the axial slices.

Isolated fiber tracts are shown in Figure 1. Fractional anisotropy (FA) and apparent diffusion coefficient (ADC) were calculated for all the tracts, separately. Also, average fiber length (in mm), fiber volume (expressed as the number of voxels occupied by each fiber tract), and fiber density (given as the mean number of fibers per voxel) was also calculated for each fiber tract. Finally, we obtained lengths of individual fibers in a fiber tract (fiber length profile) and compared the profiles of each fiber tract between the ASD group and TD group.

Tractography was performed independently by two investigators (A.K. and S.K.S.) who were masked to the subject groups. The interrater reliability was assessed by determining the correlation between the observers. The overall correlation coefficient of the measurements between observers was 0.94 ($P < 0.01$).

Tract-Based Spatial Statistics

Tract-based spatial statistics (TBSS, Analysis Group, FMRIB, Oxford, UK), an automated, operator-independent voxelwise analysis of brain white matter, was also performed for FA and ADC (Smith et al. 2006). TBSS projects all subjects' FA data onto a mean FA tract skeleton, before applying voxelwise cross-subject statistics. In brief, all subjects' FA data were aligned into a common space using the nonlinear registration tool FNIRT, which uses a *b*-spline representation of the registration warp field. Next, the mean FA image was created and thinned to create a mean FA skeleton that represents the centers of all tracts common to the group. Each subject's aligned FA data were then projected onto this skeleton, and the resulting data were fed into voxelwise cross-subject statistics. In a separate process using the FA image–derived skeleton, the maximum values along the direction perpendicular to the tract of the ADC image are projected to a separate skeleton image. Voxelwise statistical analysis of individual skeleton images of all subjects was performed between ASD, DI, and TD groups, for FA and ADC separately, using a nonparametric permutation test with a cluster size threshold of >3 and *P* value of <0.05 for significance, after correcting for multiple comparisons.

Statistical Analysis

Values are reported as mean ± standard deviation (SD). Separate 3 (group) × 2 (hemisphere) repeated measures analysis of variance (ANOVA), followed by post-hoc analysis for multiple comparisons, was performed to evaluate between-group differences for each parameter for all the fiber tracts, except the CC, which was analyzed using univariate ANOVA. Group × hemisphere analysis was also used to determine the differences in the patterns of asymmetry between groups. This was followed by simple effect test, using ANOVA with post-hoc analysis for multiple comparisons, to see the difference in individual tracts between groups.

The DTI parameters were also correlated with developmental and behavioral variables (VABS communication, GARS AQ, GARS stereotypic behavior, and GARS social isolation) within ASD or DI groups. For these analyses, age was used as a covariate, and partial Pearson correlation coefficients were obtained. SPSS 17.0 was used for the statistical analysis.

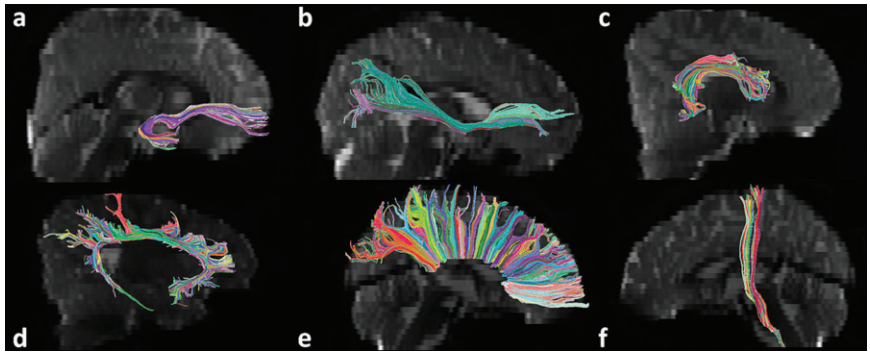

Figure 1. Image showing (*a*) UF, (*b*) IFO, (*c*) AF, (*d*) Cg, (*e*) CC, and (*f*) CST.

Results

Diffusion Parameters (FA and ADC)

The mean values of FA and ADC for all the tracts are given in Table 2. FA was significantly lower in right UF ($P = 0.03$), left AF ($P = 0.01$), right Cg ($P = 0.04$), and CC ($P = 0.04$) in ASD group and in right UF ($P = 0.001$), bilateral IFO (left: $P = 0.01$, right: $P = 0.008$), right Cg ($P = 0.01$), and CC ($P = 0.03$) in DI group, compared with the TD group. Right AF was found to have higher diffusivity (ADC) in both ASD group ($P = 0.03$) and DI group ($P = 0.003$), compared with the TD group. No difference in FA or ADC of any of the fiber tracts was found between the ASD and DI group.

Three separate TBSS analyses (comparing TD group vs. ASD group or DI group and ASD group vs. DI group) showed that similar areas were affected in ASD or DI children. Voxels in the regions of bilateral UF, IFO, AF, right Cg, and CC had significantly lower FA in both groups compared with TD group (Fig. 2). No difference in ADC was found between TD group and the ASD or DI group. Similar to the tractography results, TBSS analysis also did not find any area of significant difference, in either FA or ADC, between ASD and DI groups.

Geometric Properties (Fiber Length, Volume, and Density)

The mean values of fiber length, fiber volume, and fiber density for all the tracts are given in Table 3. The ASD group showed shorter fiber length in left UF ($P = 0.008$) but longer fiber length ($P = 0.04$), increased fiber volume ($P = 0.006$), and higher fiber density ($P = 0.001$) in right UF, compared with the TD group. The ASD group also had longer fiber length ($P = 0.008$) and higher fiber density ($P = 0.03$) of CC and higher fiber density ($P = 0.02$) of the left Cg, compared with the TD group. The DI group had significantly shorter fiber length of left UF ($P = 0.04$) and right IFO ($P = 0.009$), compared with the TD group. Compared with the DI group, ASD group had higher fiber length ($P = 0.003$), higher fiber volume ($P = 0.01$), and increased fiber density ($P = 0.005$) in right UF; higher fiber volume ($P = 0.009$) of left UF; and increased fiber length of right AF ($P = 0.001$) and CC ($P = 0.04$).

Table 2
Diffusion parameters for different fiber tracts

Tract	Sides	Subjects	FA	*P* value		ADC	*P* value	
UF	Left	TD	0.46 ± 0.04	0.11*	0.21†	2.41 ± 0.13	0.16	0.49
		ASD	0.44 ± 0.02		0.04‡	2.60 ± 0.14		0.06
		DI	0.42 ± 0.03		0.21!	2.78 ± 0.13		0.13
	Right	TD	0.46 ± 0.04	0.006	0.03	2.43 ± 0.14	0.06	0.18
		ASD	0.43 ± 0.03		0.001	2.61 ± 0.26		0.02
		DI	0.41 ± 0.02		0.05	2.78 ± 0.3		0.13
IFO	Left	TD	0.52 ± 0.04	0.04	0.11	2.38 ± 0.13	0.43	0.28
		ASD	0.49 ± 0.03		0.01	2.55 ± 0.13		0.22
		DI	0.48 ± 0.03		0.13	2.60 ± 0.14		0.70
	Right	TD	0.52 ± 0.04	0.03	0.11	2.36 ± 0.10	0.23	0.16
		ASD	0.50 ± 0.03		0.008	2.55 ± 0.13		0.11
		DI	0.48 ± 0.03		0.10	2.61 ± 0.14		0.56
AF	Left	TD	0.50 ± 0.04	0.04	0.01	2.26 ± 0.12	0.12	0.12
		ASD	0.46 ± 0.03		0.06	2.46 ± 0.13		0.05
		DI	0.47 ± 0.03		0.97	2.57 + 0.14		0.38
	Right	TD	0.49 ± 0.03	0.24	0.93	2.24 ± 0.06	0.01	0.03
		ASD	0.47 ± 0.04		0.13	2.5 ± 0.13		0.003
		DI	0.46 ± 0.02		0.11	2.7 ± 0.14		0.08
Cg	Left	TD	0.48 ± 0.04	0.06	0.41	2.30 ± 0.19	0.37	0.30
		ASD	0.46 ± 0.03		0.03	2.46 ± 0.15		0.17
		DI	0.44 ± 0.02		0.06	2.53 ± 0.16		0.54
	Right	TD	0.47 ± 0.04	0.04	0.04	2.28 ± 0.17	0.21	0.19
		ASD	0.44 ± 0.03		0.01	2.46 ± 0.15		0.09
		DI	0.43 ± 0.03		0.24	2.54 ± 0.15		0.44
CST	Left	TD	0.62 ± 0.03	0.16	0.11	2.11 ± 0.11	0.42	0.42
		ASD	0.60 ± 0.05		0.08	2.2 ± 0.13		0.18
		DI	0.59 ± 0.04		0.21	2.3 ± 0.12		0.43
	Right	TD	0.60 ± 0.04	0.48	0.26	2.11 ± 0.10	0.38	0.80
		ASD	0.58 ± 0.04		0.33	2.12 ± 0.11		0.30
		DI	0.58 ± 0.03		0.96	2.31 ± 0.12		0.17
CC		TD	0.57 ± 0.03	0.04	0.04	2.49 ± 0.14	0.45	0.41
		ASD	0.54 ± 0.02		0.03	2.63 ± 0.13		0.21
		DI	0.53 ± 0.02		0.47	2.71 ± 0.14		0.49

Note: TD, typically developing group; ASD, autism spectrum disorder group; DI, nonautistic developmentally impaired group. All values are given as mean ± SD (no unit for FA and 10^{-3} mm/s^2 for ADC). *P* values are taken from ANOVA followed by post-hoc analysis: *overall; †between TD and ASD groups; ‡between TD and DI groups;!between ASD and DI groups.

Asymmetry Analysis

Group × side repeated measures ANOVA was used to determine the difference in the pattern of asymmetry between ASD, DI, and control children. There was a reversed pattern of asymmetry in average fiber length ($P = 0.004$, Fig. 3*a*) and fiber density ($P = 0.01$, Fig. 3*b*) of the UF and average fiber length ($P = 0.01$, Fig. 3*c*) of the AF in the ASD group compared with the TD and DI groups. In other words, average fiber length and fiber density of the UF and average fiber length of AF was higher in the right hemisphere in the ASD group, whereas this pattern was opposite in TD and DI children.

Analysis of Fiber Length Profile

Histograms of fiber length were plotted for each tract in order to visualize differences between groups. Although the histogram of right UF length showed a bimodal distribution in all the groups, the second peak was distinctly larger in the ASD group (skewed to the left $P = 0.006$), indicating a greater number of long fibers (Fig. 4*a*). The right AF had a bimodal distribution in the TD group, whereas it was unimodal in both ASD and DI groups. Furthermore, it was more right skewed in the ASD group ($P = 0.01$), indicating more long fibers (Fig. 4*b*). Significant group × side interactions for skewness ($P = 0.002$ for UF and 0.007 for AF) in repeated measure ANOVA were also obtained.

There was no difference in the distribution profile of fiber length of IFO, Cg, and CST. Although there was no statistical difference in fiber length profile of CC, the peak in the ASD group appeared to be shifted to the right by 5–10 pixels, indicating a higher number of longer fibers.

In order to better understand how the uncinate fiber tracks coursed to achieve longer length in the ASD group, the tracks generated were superimposed on each child's own MRI (Fig. 5). Upon visual inspection, the right UF in ASD children showed a different shape than in the TD or DI children. The tract appeared to course beyond the temporal lobe and then turned sharply into the temporal cortex.

Correlation with Neuropsychological Data

There was a significant positive correlation between fiber volume of the left UF and GARS AQ ($r = 0.80$, $P = 0.01$), GARS stereotypic behavior ($r = 0.66$, $P = 0.07$) and GARS social isolation ($r = 0.86$, $P = 0.007$), and fiber density of left UF and GARS stereotypic behavior ($r = 0.74$, $P = 0.03$), and GARS social isolation ($r = 0.77$, $P = 0.02$). Similarly, fiber volume of right UF ($r = 0.83$, $P = 0.01$) and fiber volume ($r = 77$, $P = 0.02$) and fiber density ($r = 0.85$, $P = 0.007$) of the right AF was also positively correlated with GARS stereotypic behavior. There was a positive correlation between fiber length and fiber density of the

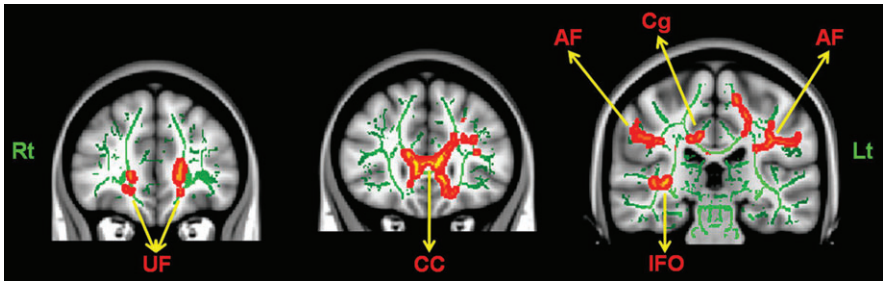

Figure 2. Results of TBSS; Coronal T_1-weighted images showing voxels with significantly lower FA value in ASD or DI children (thick red), superimposed over FA skeleton (green).

Table 3
Fiber length, fiber tract volume, and fiber density for all the fiber tracts

Tract	Sides		Average fiber length (mm)	*P* value		Fiber volume (voxels)	*P* value		Fiber density (fiber/voxel)	*P* value	
UF	Left	TD	81 ± 13	0.03*	0.01†	1519 ± 465	0.02	0.15	12.0 ± 4.2	0.22	0.97
		ASD	70 ± 13		0.04‡	1862 ± 857		0.24	11.9 ± 5.1		0.14
		DI	69 ± 15		0.92¦	1177 ± 671		0.009	9.3 ± 4.1		0.10
	Right	TD	69 ± 15	0.008	0.04	1406 ± 619	0.005	0.006	8.3 ± 4.3	0.001	0.001
		ASD	78 ± 11		0.26	2008 ± 690		0.98	13.7 ± 5.4		0.91
		DI	64 ± 14		0.003	1400 ± 577		0.01	8.5 ± 4.0		0.005
IFO	Left	TD	127 ± 16	0.11	0.94	3783 ± 1766	0.44	0.81	21.0 ± 8.7	0.81	0.88
		ASD	128 ± 14		0.09	3901 ± 1556		0.36	20.6 ± 8.7		0.55
		DI	117 ± 17		0.04	3222 ± 1318		0.20	19 ± 8.7		0.58
	Right	TD	124 ± 12	0.003	0.64	4116 ± 1408	0.61	0.64	24.6 ± 8.9	0.78	0.87
		ASD	125 ± 11		0.009	3911 ± 1451		0.32	25.0 ± 8.9		0.64
		DI	112 ± 10		0.001	3573 ± 1152		0.47	23.1 ± 5.5		0.49
AF	Left	TD	89 ± 12	0.57	0.91	1726 ± 889	0.75	0.86	13.5 ± 6.4	0.55	0.56
		ASD	89 ± 11		0.41	1669 ± 1114		0.47	14.5 ± 4.7		0.64
		DI	85 ± 13.9		0.30	1425 ± 827		0.51	12.5 ± 6.3		0.29
	Right	TD	89 ± 6	0.004	0.1	1549 ± 999	0.46	0.43	13.4 ± 5.6	0.8	0.70
		ASD	96 ± 12		0.07	1883 ± 1064		0.69	14.6 ± 7.7		0.79
		DI	78 ± 4		0.001	1317 ± 751		0.27	12.4 ± 7.8		0.53
Cg	Left	TD	37 ± 6	0.09	0.11	3725 ± 1670	0.34	0.74	13.4 ± 3.4	0.04	0.02
		ASD	42 ± 11		0.72	3580 ± 1333		0.17	16.3 ± 4.4		0.80
		DI	36 ± 8		0.05	2986 ± 1008		0.20	13.8 ± 3.4		0.07
	Right	TD	37 ± 9	0.27	0.63	3989 ± 1730	0.07	0.58	13.3 ± 4.6	0.35	0.26
		ASD	38 ± 7		0.30	3741 ± 1415		0.03	14.8 ± 3.9		0.92
		DI	34 ± 6		0.10	2770 ± 822		0.04	13.2 ± 2.8		0.23
CST	Left	TD	95 ± 7	0.17	0.08	613 ± 234	0.16	0.25	5.7 ± 2.6	0.22	0.57
		ASD	100 ± 10		0.11	740 ± 347		0.11	6.2 ± 3.2		0.09
		DI	99 ± 9		0.87	843 ± 371		0.17	7.8 ± 3.6		0.16
	Right	TD	99 ± 15	0.47	0.42	551 ± 252	0.23	0.53	5.1 ± 2.9	0.28	0.62
		ASD	103 ± 13		0.23	610 ± 269		0.09	5.6 ± 2.8		0.12
		DI	106 ± 16		0.55	743 ± 335		0.20	6.9 ± 2.9		0.20
CC		TD	76 ± 7	0.02	0.01	31305 ± 10438	0.15	0.96	34.1 ± 3.8	0.07	0.03
		ASD	83 ± 9		0.62	31422 ± 7068		0.11	37.9 ± 6.2		0.66
		DI	78 ± 6		0.04	26484 ± 5059		0.05	35.1 ± 5.1		0.13

Note: TD, typically developing group, ASD, autism spectrum disorder group, DI, nonautistic developmentally impaired group. All values are given as mean ± SD. *P* values are taken from ANOVA followed by post-hoc analysis: *Overall; †between TD and ASD groups; ‡between TD and DI groups;¦between ASD and DI groups.

CC and VABS communication (r = 0.69, P = 0.006; r = 0.83, P =0.001, respectively).

Discussion

The major findings of the present study are that the frontal lobe tracts and CC show specific alterations in diffusion or geometric properties of the tracts in children with ASD compared with DI and TD children using both tractography and TBSS approaches. DI children also showed differences compared with the TD children. However, the specific tracts involved and the form of the abnormality differed between the ASD and DI groups. Whereas, the right UF, right Cg, right AF, and CC had altered microstructural integrity (lower FA or higher ADC) in both of the groups; left AF had specifically altered microstructural integrity in ASD children and bilateral IFO had altered microstructural integrity in DI children. Similarly, whereas both ASD and DI children had shorter fiber length of left UF, only ASD children had longer fiber length, increased fiber volume, and higher fiber density in right UF; longer fiber length with higher fiber density of CC; and higher fiber density of the left Cg. DI children had specifically shorter fiber length of right IFO. When directly compared with DI group, ASD children had longer fiber length, higher fiber volume, and higher fiber density in right UF and increased fiber length of right AF.

The present study not only reconfirmed our finding of longer frontal lobe association fibers in ASD children from our previous study (Sundaram et al. 2008b), it further identified the particular fiber tracts (i.e., right UF and right AF) responsible for this finding. Previously reported increase in frontal white matter volume in ASD children also appears to be associated with specific tracts (bilateral UF), and right hemisphere seems to be more involved. Tracts showing alterations in the ASD group are

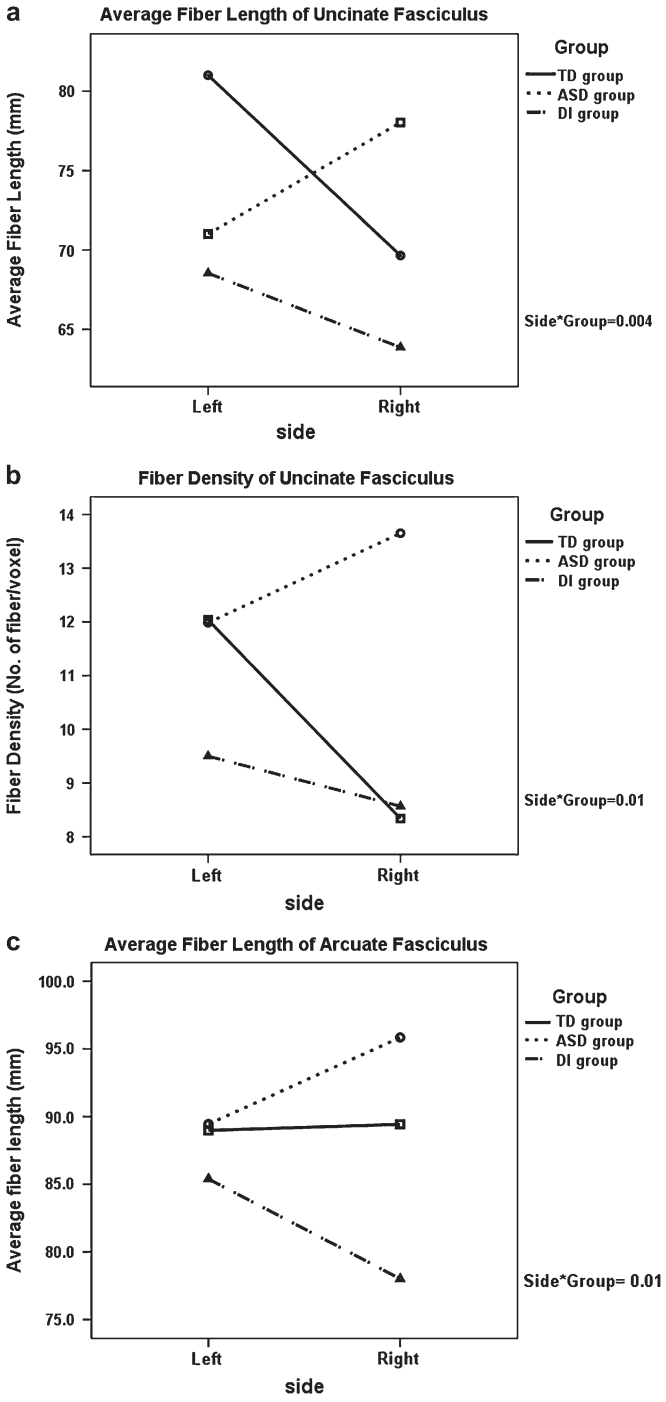

Figure 3. Plots of (*a*) average fiber length and (*b*) fiber density of the UF, and (*c*) average fiber length of the AF showing a reversed pattern of asymmetry in children with ASD compared with TD and DI children.

those known to be involved in socio-emotional and language functions, and geometric white matter abnormalities in fronto-temporal tracts appeared to be associated with magnitude of autistic symptoms.

Mechanisms of Changes in Diffusion and Geometric Parameters and Their Implications

DTI is a very sensitive measure of white matter maturation. Abnormalities in myelination, axonal number, diameter, and orientation can all lead to changes in FA and ADC (Basser 1995). The relative sensitivity of the various diffusion parameters in identifying abnormalities may be dependent upon the underlying pathology. For example, if there is a change in diffusion in all directions, such as in stroke or cell death, ADC is likely to be a very sensitive measure as it represents the average of diffusivities along three main directions (*x*, *y*, and *z*). This averaging process tends to smooth a change in diffusivity along any particular direction. On the other hand, a change in diffusivity along one particular direction (i.e., along the fibers or perpendicular to it), such as change in myelination or in the case of increased axonal swelling or increased intercellular space, tends to magnify the change in FA. As the diffusion anisotropy increases, the FA measurement is likely to be the more sensitive parameter in identifying such changes. Because the specific white matter tracts studied in the present study are highly anisotropic, FA is likely to be more sensitive than ADC in identification of abnormalities. This perhaps explains the lack of any significant difference in ADC, except for right AF, even though significant difference in FA was found for several tracts.

The major change in FA in ASD and DI subjects in the present study suggests either a reduced diffusivity along the fiber pathway or a change in diffusivity in the directions perpendicular to the fibers. There may be several reasons for these changes: 1) increased intercellular space (intercellular space can increase if there are fewer axons or if the axons are thinner than normal), 2) increased intracellular volume (i.e., increased axonal width or axonal swelling), or 3) decreased or abnormal myelination. The reason for increased intercellular space, leading to reduced FA, was probably thinner, but numerous axons in the ASD group, further corroborated by increased fiber density and volume in this group. On the other hand, reason for increased intercellular space in the DI group may be reduced number of fibers, which was further corroborated by decreased fiber density in these children compared with the ASD group. This increase in volume and density but decreased functionality (connectivity), particularly in the ASD children, may be because of large number of remaining unpruned axons leading to higher cross-connection and increased noise, which may lead to inefficient signal transmission. Fiber volume of bilateral UF and right AF and fiber density of left UF were found to be positively associated with stereotypic behavior, social isolation, and overall autistic triad symptoms, that is, children with higher volume of these fiber tracts had increased symptoms. Fiber length and fiber density of CC were found to be positively associated with functional developmental. It may be possible that more number of fibers (increased density) and increased fiber length may have been an effort to enhance interhemispheric connectivity to achieve better functional integration and development.

Previous DTI Studies

In the first report of DTI in autism, Barnea-Goraly et al. (2004) found reduced FA values in left fusiform gyrus, superior temporal sulcus, ventromedial prefrontal cortex, bilateral anterior cingulate, bilateral temporoparietal junction, superior temporal sulcus, and bilateral amygdala in autistic subjects using statistical parametric mapping (SPM) analysis. Another SPM study showed reduced FA in CC (genu, posterior midbody), right anterior corona radiata, and right retrolenticular portion of internal capsule (Keller et al. 2007). Using an "ROI approach," Alexander et al. (2007) showed reduced FA, increased ADC, and

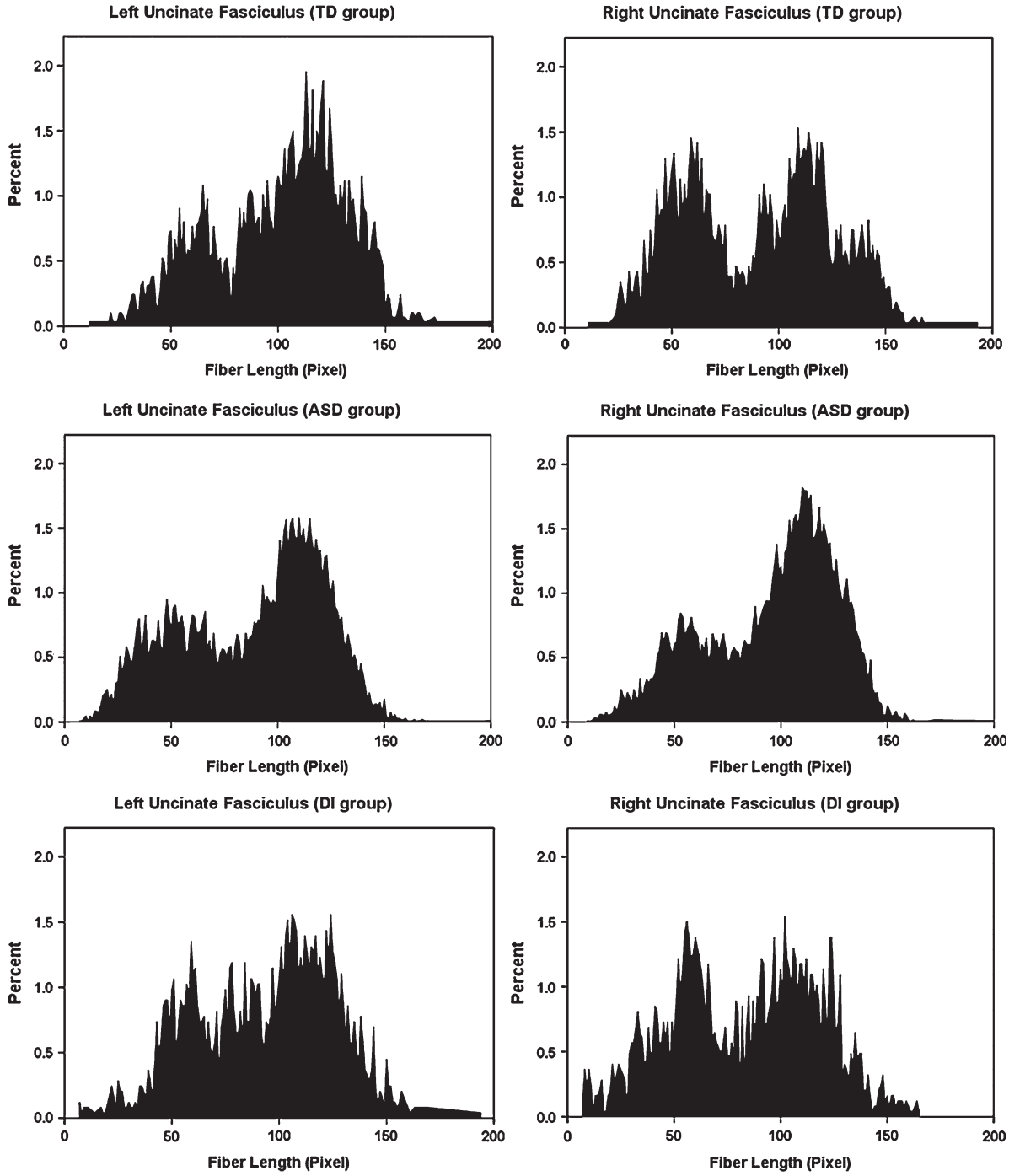

Figure 4. Histograms showing fiber length profiles of (*a*) UF and (*b*) AF in TD, ASD, and DI groups. Although the histogram of right UF length showed a bimodal distribution in all the groups, the second peak was distinctly larger in the ASD group (skewed to the right, $P = 0.006$), indicating a greater number of long fibers. The right AF in TD group also had a bimodal distribution. However, it was unimodal in both ASD and DI groups, but much more right skewed in the ASD group ($P = 0.01$), indicating more long fibers, in the ASD group.

increased RD in multiple regions of the CC. Lee et al. (2007), using white matter segmentation of superior temporal gyrus and temporal stem, found decreased FA and increased diffusivity in the superior temporal gyrus and temporal stem in autistic patients. Using overall white matter pixel counts and ROI approach, Ben Bashat et al. (2007) found early and accelerated abnormal maturation of white matter in very young children with autism (age: 1.8–3.3 years), particularly involving frontal lobe and Thakkar et al. (2008) evaluated anterior Cg in ASD children and found reduced FA in bilateral anterior Cg. Catani et al. (2008) found significantly lower FA in the short intracerebellar fibers and right superior cerebellar (output) peduncle in patients with Asperger syndrome. Recently, Ke et al. (2009) reported decreased FA in the frontal lobe and left temporal lobe in a group of Chinese children with high functioning autism. These findings are consistent with our results, which also showed reduced FA and increased diffusivity (ADC) in different fiber tracts.

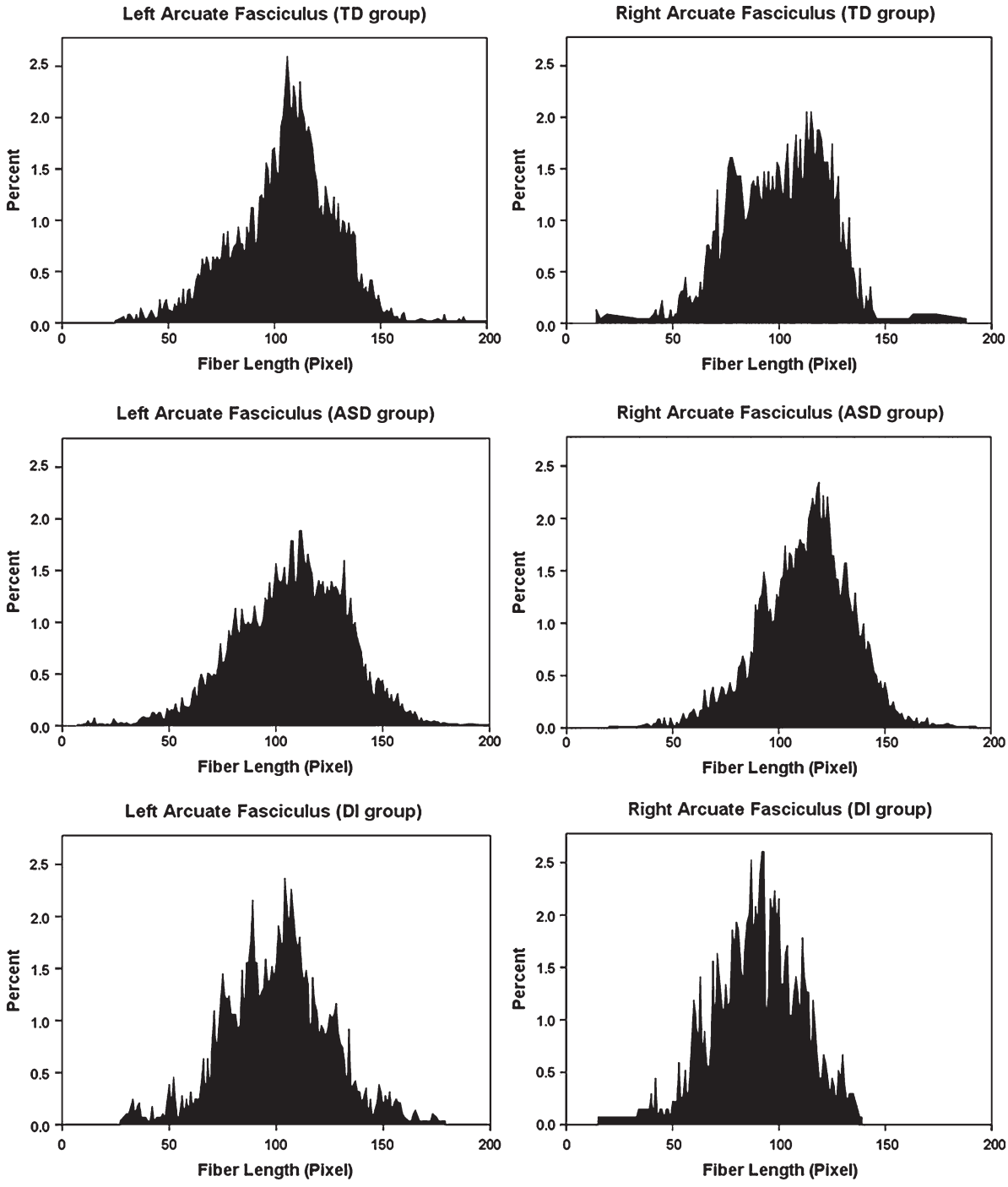

Figure 4. Continued.

Although there are only few DTI studies in nonautistic developmentally impaired children, they have found white matter abnormalities in these children. Filippi et al. (2003) reported decreased FA in the region of central semiovale, corona radiate, CC, and subcortical white matter of the frontal and parieto-occipital lobes. Another study found reduced FA and decreased volume of CC (Ding et al. 2009). Previous DTI study from our center (Sundaram et al. 2008b), only study to evaluate major association tracts using tractography approach in children with global developmental delay of unknown etiology, found reduced FA in UF, IFO, and Cg, similar to our present findings.

Abnormal Connectivity in Autism

A number of recent studies have suggested that autism is a disorder of cortical networks rather than associated with dysfunction in discrete cortical regions (Just et al. 2004; Bachevalier and Loveland 2006; Just et al. 2007; Minshew and Williams 2007; Muller 2007; Rippon et al. 2007). While a cortical abnormality may result in functional impairment specific to that region, abnormal cortical connectivity will result in the impairment in integrating the functions of the affected cortical regions. From this perspective, the cognitive deficit in autism involves inability to bind together a collection of separate

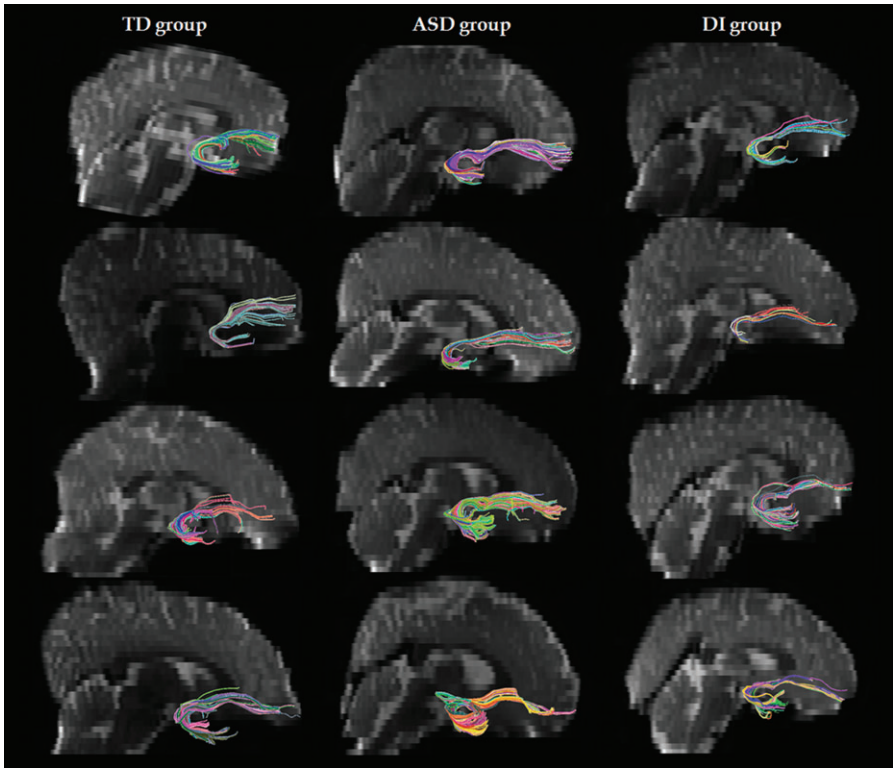

Figure 5. Right UF in 4 TD children (TD group, left panel), 4 ASD children (ASD group, middle panel), and 4 nonautistic developmentally impaired children (DI group, right panel). The UF in ASD children, compared with DI and TD children is longer and appears to course beyond the temporal lobe and then turns more sharply to end in temporal cortex.

features into a coherent concept even though their ability to analyze individual features may be preserved (Frith 1989). Several studies suggest that this functional underconnectivity in autism involves language, social cognition (Castelli et al. 2002), working memory (Koshino et al. 2005), and problem solving (Just et al. 2007).

For example, abnormal social cognition is one of the core features of autism. The amygdalo-orbitofrontal circuit is necessary for appropriate development of social cognition, and its involvement has been implicated in autism (Bachevalier and Loveland 2006). The fibers connecting amygdala and orbitofrontal cortex form a significant fraction of UF. Therefore, the DTI abnormalities demonstrated in the present study are consistent with an aberrant amygdalo-orbitofrontal circuit. This circuit also feeds into the fronto-striatal pathway, which has been implicated in the stereotypic behavior of autism (Nordahl et al. 2007). Therefore, abnormality of amygdalo-orbitofrontal circuit may in turn affect the functioning of fronto-striatal pathway. This may explain the association between DTI findings in UF and stereotypic behavior observed in the present study.

Similarly, involvement of the AF, found in our study, is consistent with language delay/deviance characteristic of autism. In fact, an fMRI study found reduced functional connectivity between Wernicke's and Broca's area in autistic children, whereas actual functions within these regions were intact (Just et al. 2004). In another study, while processing of single words (a low-level task) was found to be well preserved in autistic children, they had impaired ability in processing the meaning of complex sentences (a high-level task requiring integration) (Goldstein et al. 1994).

Cingulate cortex dysfunction, particularly of anterior cingulate, has been linked to indices of social impairment (Haznedar et al. 2000; Ohnishi et al. 2000; Kennedy et al. 2006) and restricted and repetitive behaviors (Shafritz et al. 2008; Thakkar et al. 2008) in ASD, and abnormality of the cingulate cortex, particularly on the right side, is suggested by various postmortem and MRI studies. Nine autistic patients studied by Bauman and Kemper showed reduced neuronal size and increased cell-packing density in the anterior cingulate gyrus (Bauman and Kemper 1985). In an MRI study of 7 autistic subjects and 7 controls, Haznedar et al. (1997) found the right anterior cingulate cortex to be significantly smaller in the autistic group. This finding was subsequently confirmed in a larger study involving 17 subjects (Haznedar et al. 2000). Involvement of the Cg in autism is also implicated by an fMRI study of working memory, which found hypoactivation of the right posterior cingulate cortex (Luna et al. 2002). Thakkar et al. (2008), recently, found increased activation and reduced FA in bilateral anterior Cg, which was related to response monitoring and repetitive behavior. Our findings, higher fiber density of the left Cg and lower FA of the right Cg in ASD group, may indicate alteration in the structural integrity of the cingulate fasciculus and are consistent with the findings cited above.

Abnormalities of the CC in autistic patients have been reported by several investigators (Alexander et al. 2007; Keller et al. 2007; Ding et al. 2009). Similar to their findings, we also found decreased FA in the CC of our ASD children. We also found the CC to have significantly longer fiber length with higher fiber density.

Methodological Considerations

Recognized methodological problems in DTI tractography include the issues of crossing fibers, kissing fibers, and reproducibility of tracking fiber tracts with complex geometry.

For example, the most medial fibers of IFO lie adjacent to the most lateral fibers of UF, in the inferior and anterior frontal white matter. Even though a small fraction of fibers may "kiss" or "cross" in their anterior end, their subsequent trajectory is distinctly different to allow adequate separation. Further, we found that the white matter region where these fibers may kiss or cross was homogeneous with respect to the DTI parameters—FA and ADC (in case of significant kissing or crossing there should have been marked change in these parameters from one voxel to another), and, therefore, this issue is unlikely to impact our results. The reproducibility of tracking algorithms to identify specific tracts depends primarily on the type of tracts. For example, it is far less reproducible to track some of the thin limbic tracts (such as stria terminalis) that approach the limit of DTI resolution compared with major association and projection tracts (Mori et al. 2005). The reproducibility of tracking association tracts on DTI has been previously validated (Mori et al. 2002; Sundaram et al. 2008a).

Use of TBSS further confirmed and strengthened our findings. TBSS is an operator independent voxel-based white matter analysis that reduces the total number of voxel comparisons and avoids the spatial smoothing used in other voxel-based techniques. We found that the regions, shown by TBSS to be significantly different in ASD or DI group (reduced FA) as compared with control group, were essentially the same as found with tractography approach. Similarly, our TBSS results were consistent with those reported by some of the previous investigators using SPM or other voxel-based approach (Barnea-Goraly et al. 2004; Ben Bashat et al. 2007; Keller et al. 2007; Lee et al. 2009).

Notes

Conflict of Interest: None declared.

Address correspondence to Ajay Kumar, MD, PhD, DNB, Assistant Professor, Department of Pediatrics and Neurology, Children's Hospital of Michigan, Detroit Medical Center, Wayne State University, School of Medicine, Detroit, MI 48201, USA. Email: ajay@pet.wayne.edu.

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