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Diffusion Tensor Imaging of Frontal Lobe in Autism Spectrum Disorder

Senthil K. Sundaram[1,2], Ajay Kumar[1,2], Malek I. Makki[2,3], Michael E. Behen[1,2], Harry T. Chugani[1,2,3] and Diane C. Chugani[1,3]

[1]Carman and Ann Adams Department of Pediatrics, [2]Departments of Neurology and [3]Radiology, Children's Hospital of Michigan, Detroit Medical Center, Wayne State University School of Medicine, Detroit, MI, 48202, USA

To investigate frontal lobe white matter in children with autism spectrum disorder (ASD), we performed diffusion tensor imaging (DTI) in 50 ASD children (mean age: 57.5 ± 29.2 months, 43 males) and 16 typically developing children (mean age: 82.1 ± 41.4 months, 11 males). The apparent diffusion coefficient (ADC) was significantly higher for whole frontal lobe ($P = 0.011$), long ($P < 0.001$) and short range ($P = 0.0126$) association fibers in ASD group. There was a trend toward statistical significance in the fractional anisotropy (FA) of whole frontal lobe fibers ($P = 0.11$). FA was significantly lower in ASD group for short range fibers ($P = 0.0031$) but not for long range fibers ($P =$ not significant [NS]). There was no between-group difference in the number of frontal lobe fibers (short and long) ($P =$ NS). The fiber length distribution was significantly more positively skewed in the normal population than in the ASD group ($P < 0.001$). The long range association fibers of frontal lobe were significantly longer in ASD group ($P = 0.026$ for both left and right hemispheres). Abnormal frontal FA and ADC may be due to white matter organization abnormalities in ASD. Lack of evidence for excessive short range connectivity in ASD in this study may need to be re-examined with future advances in DTI technology.

Keywords: apparent diffusion coefficient, fractional anisotropy, magnetic resonance imaging, short range connectivity, tractography

Introduction

Autism is a behaviorally defined disorder with core deficits in social interaction and communication as well as stereotypic behaviors. Social deficits are often the earliest deficits in autism and there is significant evidence to suggest that the social deficits may be related to a social cognition network in which frontal lobe plays a crucial role (Baron-Cohen et al. 1999). This notion is supported by a number of structural and functional neuroimaging studies, which have found frontal lobe abnormalities in patients with autism (Carper and Courchesne 2000, 2005; Chandana et al. 2005; Chugani et al. 1997, 1999; Hazlett et al. 2004; Herbert et al. 2003, 2004; Luna et al. 2002; Ohnishi et al. 2000; Salmond et al. 2003). Carper et al found that the greatest increase of gray and white matter volume occurred in the frontal lobe (Carper et al. 2002), a finding which was subsequently reproduced by others (Herbert et al. 2004). Similarly, a functional magnetic resonance imaging (fMRI) study of spatial working memory task in 11 high functioning autistic children and 6 healthy volunteers demonstrated that there was significantly less task-related activation in dorsolateral prefrontal cortex and anterior cingulate cortex in the autistic group (Luna et al. 2002). An [18F]fluoro deoxy glucose positron emission tomography study of regional glucose metabolism in autistic and control subjects showed a reduced glucose metabolic rate in the medial frontal cortex associated with greater perseverative/intrusion errors in the autistic group (Hazlett et al. 2004). In our previous studies of serotonin synthesis using PET in autistic subjects, we also reported significant abnormalities in frontal lobe (Chandana et al. 2005; Chugani et al. 1999). We 1st observed decreased serotonin synthesis unilaterally in dorsolateral prefrontal cortex in 7 boys (Chugani et al. 1999) but, in a larger study, we found both unilateral and bilateral decreases of serotonin synthesis capacity affecting varying extents of the frontal lobe (Chandana et al. 2005). Because serotonin acts early in life as a neurotrophic factor affecting thalamocortical axonal arborization (Gaspar et al. 2003), we hypothesized that altered frontal lobe serotonin synthesis capacity in autistic children may be related to abnormalities in underlying connectivity of frontal lobe. Abnormalities documented by neuroimaging in different prefrontal cortical areas have been found to correlate with deficits of social cognition, executive functioning and language in autism (Abell et al. 1999; Castelli et al. 2002; Ernst et al. 1997; Luna et al. 2002). These brain–behavior correlation studies suggest a strong involvement of frontal lobe in the expression of different manifestations of autism.

A number of recent studies suggest that autism is a disorder of cortical networks rather than associated with dysfunction in discrete cortical regions (Bachevalier and Loveland, 2006; Just et al. 2004). In autism, this network abnormality manifests as functional underconnectivity between different cortical regions. Although a regional cortical abnormality is expected to result in impairment of task performance of that region, underconnectivity of cortical regions is likely to result in impairment in the integration of tasks. From this perspective, the cognitive deficit in autism involves inability to bind together a collection of separate features into a coherent concept even though their ability to analyze individual features may be preserved (Frith 1989). Consistent with this model, Goldstein et al showed that high functioning autistic children have unusual strength in processing single words (a low level task) while being impaired in their ability to process the meaning of complex sentences (a high level task requiring integration) (Goldstein et al. 1994). This issue was further explored in an fMRI study of written sentence comprehension that showed reduced functional connectivity between Wernicke's area and Broca's area in high functioning autistic children even though activation in Wernicke's area, a region necessary for processing single words, is enhanced (Just et al. 2004). Several studies suggest that this functional underconnectivity in autism involves not only language, but also social cognition (Castelli et al. 2002), working memory (Koshino et al. 2005) and problem solving (Just et al. 2007).

Findings from conventional structural and functional MRI studies suggest the involvement of specific cortical networks based on the anatomic patterns of abnormalities, but do not *directly* demonstrate the abnormalities in the networks themselves. Diffusion tensor imaging (DTI) can provide additional information about the structure of cortical networks. DTI relies on the pattern of diffusion of water molecules to provide information about the architecture of white matter. Different quantitative measures can be derived from a DTI study, such as the degree of restriction to water diffusion quantified by the parameter "apparent diffusion coefficient" (ADC) and the directionality of water diffusion quantified by the parameter "fractional anisotropy" (FA). DTI has been shown to be an extremely sensitive measure of white matter maturation, especially in comparison to other MRI measures such as T1 and T2 relaxation times (Baratti et al. 1999; Huppi et al. 1998; Neil et al. 1998). For example, abnormalities in myelination, axonal number, diameter and orientation can all lead to changes in FA and ADC (Beaulieu, 2002). To date 3 DTI studies of autism have been published (Alexander et al. 2007; Barnea-Goraly et al. 2004; Keller et al. 2007). Using statistical parametric mapping (SPM), Barnea-Goraly et al. found reduced FA values in brain regions that were implicated in social cognition (fusiform gyrus and superior temporal sulcus) and in theory of mind tasks (ventromedial prefrontal cortex, anterior cingulate, temporoparietal junction, superior temporal sulcus, and amygdala) (Barnea-Goraly et al. 2004). Similarly, another DTI study in autistic subjects showed reduced FA in corpus callosum (genu, posterior midbody), right anterior corona radiata and right retrolenticular portion of internal capsule (Keller et al. 2007). The corpus callosum alone was examined in detail in another study by Alexander et al. (2007) who showed reduced FA, increased mean diffusivity and increased radial diffusivity in autistic patients (Alexander et al. 2007). These microstructural abnormalities were found to be related to nonverbal cognitive performance. Several studies of autistic subjects have suggested that there is excessive short range connectivity in frontal lobe, but its structural basis has not been directly demonstrated (Belmonte et al. 2004; Casanova et al. 2002; Herbert et al. 2004). The current study was designed to determine whether DTI analyzed with tractography methods can further elucidate white matter abnormalities in frontal lobe and to test whether there are changes in short range connectivity in children with autism spectrum disorder (ASD). Accordingly, the purpose of the current study was to further test the hypothesis that there is excessive short range connectivity, low anisotropy and high diffusivity of frontal lobe fibers in children with ASD.

Materials and Methods

Subjects

We selected consecutively fifty children with a diagnosis of ASD (mean age: 57.5 ± 29.2 months, 43 males, 7 females) who had DTI performed as part of the clinical MRI protocol (Table 1). Inclusion criteria for the ASD group included a diagnosis of autism, Asperger's Disorder, or pervasive developmental disorder not otherwise specified (PDD-NOS) according to Diagnostic and Statistical Manual of Mental Disorders 4th Edition (DSM-IV) criteria, scores equal to or greater than 15 on the Social Communication Questionnaire (SCQ) (Rutter et al. 2003) and scores on the Autism Quotient of the Gilliam Autism Rating Scales (GARS) (Gilliam, 1995) of 80 or above. Participants were children with previous diagnoses made by pediatric neurologists using DSM-IV TR criteria. These children were identified and subsequently followed up and underwent neurodevelopmental and behavioral screening. No attempt was made to distinguish between different diagnostic groups (autism, Asperger's disorder and PDD-NOS). The SCQ was used to screen for the presence of an ASD, and GARS was used to measure the magnitude of triad symptoms in order to ensure the presence of substantial autistic symptoms. Thus, the purpose of these inclusion criteria was to ensure that children met criteria for an ASD and had significant autistic spectrum symptoms. Only right handed children were included in the current study. Eleven of the patients were on risperidone, and 2 were on buspirone at the time of DTI acquisition. Because the scans for children with ASD were clinical MRI studies, sedation was used as necessary by the sedation team at Children's Hospital of Michigan. Children with the following characteristics were excluded from the study: 1) history of seizures, 2) diagnosis of nonautistic developmental delay/mental retardation, 3) focal deficits on clinical examination by a pediatric neurologist, 4) MRI interpreted as abnormal by a pediatric neuroradiologist, 5) dysmorphic features suggestive of a genetic syndrome, 6) an inborn error of metabolism, 7) history of prematurity or perinatal hypoxic-ischemic event. The Human Investigations Committee at Wayne State University granted permission for the retrieval and analysis of de-identified MRI data that had been obtained clinically for these children.

Sixteen typically developing children (mean age: 82.1 ± 41.4 months, 11 males and 5 females) were also studied with MRI including DTI as a control group. All the typically developing children were screened by a neurologist and a pediatric neuropsychologist and none of them had any historical or current medical, developmental, or psychiatric diagnoses. None of these children were sedated for the MRI. The participants were studied according to 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 and a written assent was obtained from children older than 12 years.

Table 1
Neuropsychological results in ASD subjects

	Mean	SD	Range
VABS adaptive behavior composite	68.25	10.60	51–96
VABS communication	66.54	12.98	44–95
VABS daily living skills	71.58	15.23	50–109
VABS socialization	72.42	17.68	51–122
VABS motor	75.25	11.67	59–107
GARS	100.95	12.57	82–130
GARS stereotypic behaviors	10.45	2.26	7–14
GARS social isolation	10.05	2.98	5–18
GARS communicative disturbances	10.35	1.50	7–13
GARS developmental disturbances	9.19	2.42	6–14

Note: VABS is a standard score with a mean of 100 and standard deviation of 15. GARS is a normalized standard score with a mean of 100 and standard deviation of 15 for the autistic population. Each of the subdomains of GARS has a mean of 10 and standard deviation of 3.

Neurobehavioral Evaluation

The neurobehavioral evaluation in the study included the Vineland Adaptive Behavior Scales, 2nd edn. (VABS) (Sparrow et al. 1984), the SCQ and the GARS. The VABS is a caregiver-reported 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. The SCQ is a caregiver-report measure based on the Autism Diagnostic Interview–Revised (Lord et al. 1994) that is widely used as a screening instrument for ASD. The measure consists of 40 items that were linked to correspond to the Diagnostic and Statistical Manual (American Psychiatric Association 2000) (Lord et al. 1994). The psychometric properties of the scale have been demonstrated to be good (Berument et al. 1999), particularly supporting the ASQ as a useful instrument for

discriminating ASD from non-ASD rather than autism from other ASD. The Gilliam Autism Rating Scale (Gilliam 1995) is a 56-item behavioral checklist that allows the quantification of frequency and severity of the triad symptoms of autism. The GARS is comprised of 4 subscales: stereotyped behaviors, communication, social interaction, and developmental disturbances, as well as an overall Autism Quotient (AQ). The AQ is a standardized score that represents the overall assessment of autistic symptoms displayed by an individual. Scores greater than 80 are considered to indicate at least a below average to high average probability of autism and indicates 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. The results of the neuropsychological evaluation of the patients with these neuropsychological tests are given in Table 1.

DTI Acquisition Protocol

MRI scans were performed using a 3-Tesla GE 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 T2 weighted ($b \simeq 0$ 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. Double refocusing pulse was used to reduce eddy-current artifacts, and array spatial sensitivity encoding technique was performed to further reduce geometric distortion due to the sequence design. Although the diffusion tensor magnetic resonance imaging (DT-MRI) sequence included double radio frequency refocusing pulses, and parallel imaging capability to reduce eddy-current and geometric distortions we did not observe major artifacts. These were not seen at the level of the deep brain structures; hence we did not perform any off line correction. The echo time was 79 ms, and the repetition time was 10 seconds. A minimum set of 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 minutes.

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 version 2.40 (May 2005, www.mristudio.org). Tractography was carried out based on Fiber Assignment by Continuous Tracking algorithm (Mori et al. 1999) with fiber propagation starting at a FA threshold value of 0.2. The fiber propagation was stopped at an FA threshold less than 0.2 or an angle threshold greater than 60°.

We initially performed tractography of the whole brain using DTI studio with the tracking parameters described above. Then approximately 50 coronal "OR" regions of interest (ROIs) (an "OR" ROI isolates the fibers passing through that ROI) were drawn starting from the frontal pole to the posterior aspect of the prefrontal cortex, in order to isolate all the fibers of prefrontal cortex. These fibers include those which remain within the frontal lobe (short association fibers) and all other fibers of the frontal lobe (long association, commissural and projection fibers) (Fig. 1). The short association fibers were further isolated by placing a "NOT" ROI (A "NOT" ROI eliminates the fibers passing through that ROI) just posterior to the last OR ROI (in the anterior premotor region).

FA, ADC, average fiber length (AVL) were calculated for all frontal fibers and short association fibers independently by 2 investigators experienced in performing tractography with DTI studio software. The average length of fibers of whole brain was also determined for both groups (i.e., ASD and control subjects) to allow direct comparison between groups and for normalization of frontal fiber length. Both investigators were blinded to the subject groups. The interrater reliability was assessed by determining the correlation between the observers. The correlation coefficient of the measurements between observers for FA was 0.96 ($P < 0.01$) and for ADC was 0.97 ($P < 0.01$), respectively.

Statistical Analysis

Two separate 3 (variables-FA, ADC, and AVL) x 2 (Group) x 2 (hemisphere) doubly multivariate analyses (MANCOVA) were performed to test for between-group differences of long and short range association fibers. Patient's age was used as a covariate in the MANCOVA tests as previous studies in typically developing children have clearly shown the age dependence of FA and ADC (Mukherjee et al. 2001; Schneider et al. 2004). Gender was also used as a covariate in the analysis. Where the overall MANCOVAs were significant, subsequent univariate repeated measures ANCOVAs were performed with group as the between-subjects factor and hemisphere as the within-subjects factor for each of the 3 outcome measures: FA, ADC, and AVL. The significance for the 2 main effects (group and hemisphere) and interaction (group × hemisphere) were examined. A *P* value of 0.05 was used as the threshold for determining statistical significance of the tests performed. Fiber length was analyzed in detail by investigating 4 moments of distribution (mean, standard deviation, skewness and kurtosis) in the 2 groups.

The DTI parameters (FA, ADC, AVL) were also correlated with neuropsychological scores obtained by VABS and GARS subscales. Age was used as a covariate and partial correlation coefficients were determined. Given the large number of comparisons, Bonferroni correction for multiple comparisons was applied.

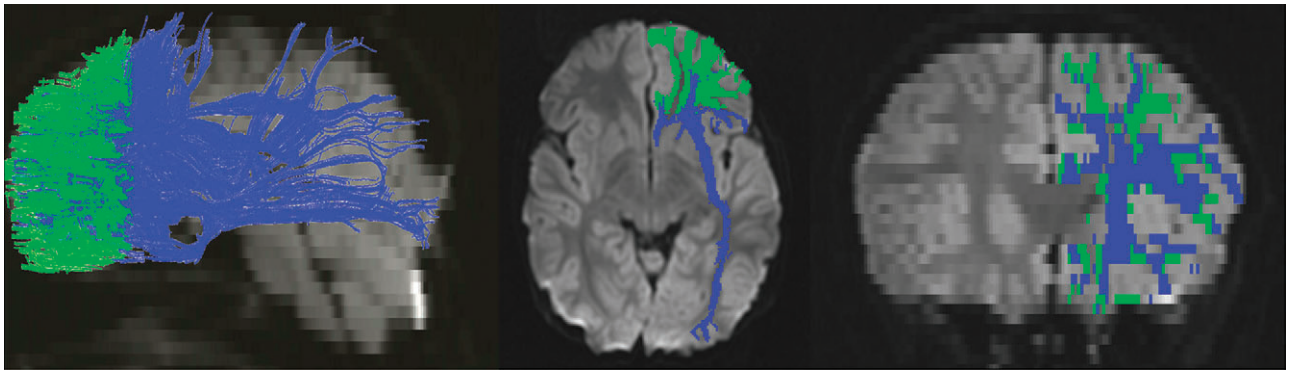

Figure 1. Long range (blue) and short range (green) association fibers of frontal lobe. Note that the tractography protocol employed in the present study is able to separate these fibers with the short fibers occupying predominantly the peripheral white matter areas close to the cortex whereas the long range fibers are predominantly localized to the central white matter. No arbitrary demarcation (as in MR volumetry) is required to separate these 2 groups of fibers. In this protocol no attempt was made to isolate the individual white matter tracts, thus circumventing the issues associated with crossing fibers.

Table 2
The estimated marginal means of FA and ADC in the study groups

	Autism		Control		*P* value
	Mean	SE	Mean	SE	
Left side					
FA					
Long range fibers	0.4602	0.0027	0.4727	0.0049	0.956
Short range fibers	0.3542	0.0024	0.3814	0.0075	**0.004**
ADC[a]					
Long range fibers	2.31	4.33	2.08	6.53	**<0.001**
Short range fibers	2.45	4.45	2.23	4.92	**0.015**
Right side					
FA					
Long range fibers	0.4597	0.0022	0.4674	0.0047	0.864
Short range fibers	0.3572	0.0024	0.3793	0.0063	**0.001**
ADC[a]					
Long range fibers	2.33	4.24	2.09	5.58	**<0.001**
Short range fibers	2.44	4.58	2.23	5.41	**0.022**

[a]Units of ADC are 10^{-3} mm^2/s for mean and 10^{-5} mm^2/s for standard error.
Statistically significant differences are shown in bold.

Results

Analysis of FA and ADC

The mean values of FA and ADC of long and short range fibers of frontal lobe are shown in Table 2. Because age was used as a covariate, the estimated marginal means (which control for age effect) of FA and ADC as determined by the MANCOVA model are provided in Table 2. The overall MANCOVAs for long and short range association fibers were highly significant ($P < 0.0001$ and $P < 0.0001$, respectively), indicating the presence of significant differences between groups in the overall dataset.

For all frontal fibers, the mean ADC in ASD children was significantly higher than that in typically developing children ($P = 0.014$). No significant difference was observed in FA of all frontal fibers between the 2 groups even though there was a trend toward significantly lower FA in the ASD group ($P = 0.132$).

For long range association fibers, the mean ADC in ASD children was significantly higher than that in typically developing children ($P < 0.001$). No significant difference was observed in FA of long frontal fibers between the 2 groups (P = NS).

For short association fibers, the mean ADC was significantly higher in the ASD group than in the typically developing children ($P = 0.015$). Similarly, the mean FA of short fibers was significantly lower in the ASD group than in the typically developing children ($P = 0.001$).

When the follow-up tests to study group differences were performed on each hemisphere, similar results were obtained, as shown in Table 2. The MANCOVA model showed that there was no group × hemisphere interaction ($P = 0.73$). Follow-up tests comparing the interhemispheric differences (after accounting for age) also showed that there was no difference between the 2 hemispheres for either FA or ADC between the ASD and the control groups.

Fiber Length Analysis

There was a highly significant positive correlation between average length of all frontal lobe fibers and the average length of fibers of whole brain ($r = 0.75$, $P < 0.0001$ for left frontal fibers and $r = 0.74$, $P < 0.0001$ for right frontal fibers). We found that there was no difference in the mean length of the fibers of whole brain ($P = 0.26$) or of the frontal lobes between the 2 groups ($P = 0.88$ for all frontal lobe fibers and $P = 0.27$ for short fibers). Even though the average length of the fibers was not different, the *distribution* of fiber length differed between the control and ASD groups. In particular, the fiber length distribution was significantly more positively skewed in the normal population than in the ASD group ($P < 0.001$). Upon visual examination, the histograms of fiber lengths showed a bimodal distribution with a smaller peak corresponding to long range fibers (Fig. 2). When the fibers corresponding to this 2nd peak were isolated, the average length of these long fibers was significantly higher in ASD than in controls ($P = 0.026$ for both left and right hemispheres). In order to examine whether there is excessive short range connectivity, we also investigated the *number* of short and long association fibers in the frontal lobe. We did not find any differences between the 2 groups ($P = 0.429$ for left hemisphere and $P = 0.217$ for right hemisphere). The number and length of fibers is summarized in Table 3.

Correlation with Neuropsychological Data

The neuropsychological evaluation showed a negative correlation between FA and GARS AQ and the social isolation subscale. However, this correlation was not significant when Bonferroni correction was applied to account for multiple comparisons. There was no other correlation between DTI variables (FA, ADC, AVL) and neuropsychological variables (VABS and its subscales, GARS and its subscales).

Discussion

The major findings of the present study are reduced FA and increased diffusivity (ADC) of the short association fibers in the ASD group compared with typically developing children. There was also significantly increased ADC of the long fibers of the frontal lobe. Although there was no difference in the average length of all frontal fibers and short association fibers between the ASD and typically developing children, the distribution of fiber length was different. Contrary to our hypothesis, we found no evidence in favor of excessive short range connectivity hypothesized to exist in subjects with autism. However, the ASD group in the current study is somewhat heterogeneous, including children diagnosed with autism, Asperger's disorder and PDD-NOS. The results of studies focusing on high functioning autism might yield different results.

Methodological Issues in Studying White Matter in Autism

Even though there are many theories of brain connectivity in autism, its structural basis has remained elusive. DTI is a promising technology which has the potential to provide important insight into the underlying structural basis of autism and also provides complementary information to other techniques such as electroencephalography, functional MRI and MR volumetry. Our results showing reduced FA and increased diffusivity ADC of the short association fibers in the ASD group compared with typically developing children, are consistent with previously reported DTI studies using SPM or ROI approaches to analysis (Alexander et al. 2007; Barnea-Goraly et al. 2004; Keller et al. 2007). The present study attempted to go beyond the SPM and ROI approaches by

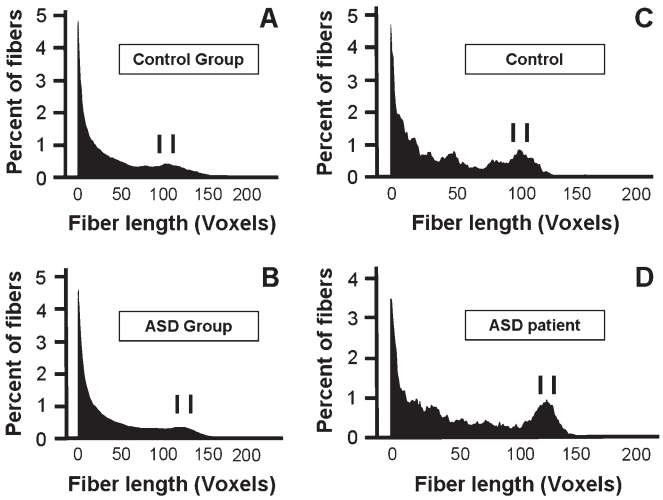

Figure 2. Histogram of fiber lengths in (*A*) the control group, (*B*) the ASD group, (*C*) a 38-month-old control child, and (*D*) a 34-month-old autistic child. The *y* axis represents the ratio of the number of fibers of given length to the total number of frontal lobe fibers (expressed as percentage). Note that the 2nd peak (located between the 2 vertical bars) is shifted to the right in the ASD group. The 2nd peak corresponds to the long range association fibers.

Table 3
Number and length of frontal lobe fibers

	Autism		Control		*P* value
	Mean	SE	Mean	SE	
Left side					
Length					
All fibers	54.41	1.36	54.15	1.82	0.92
Short range fibers	34.07	0.51	34.34	0.63	0.78
Long range fibers	120.31	0.42	118.38	0.75	**0.026**
Number					
All fibers	21 451	831	19 219	1140	0.165
Short range fibers	16 973	631	15 347	942	0.189
Long range fibers	2984	171	2711	293	0.429
Right side					
Length					
All fibers	56.41	1.57	54.84	2.34	0.62
Short range fibers	35.41	0.56	35.25	0.84	0.89
Long range fibers	120.32	0.44	118.20	0.84	**0.026**
Number					
All fibers	22 351	766	20 375	1237	0.21
Short range fibers	17 439	551	16 277	1009	0.31
Long range fibers	3313	210	2782	344	0.217

Statistically significant differences are shown in bold.

employing a diffusion tractography approach. As can be seen in Figure 1, the short range fibers occupy the peripheral regions of white matter adjacent to cortex and can be nicely separated out from long range fibers which occupy a more central portion of the white matter. The tractographic protocols employed in the present study could discriminate between the short and long range fibers and obviate the need for an arbitrary demarcation as used in earlier studies (Herbert et al. 2004).

Mechanisms of White Matter Abnormalities in Autism

Several different mechanisms can contribute to reduced frontal lobe FA and increased ADC in patients with autism. Decreased myelination, decreased axonal density, and abnormal axonal organization are some of the important mechanisms which can cause decreased FA and increased ADC. In postmortem studies on 8 well-documented postmortem autistic brains, myelination was found to be comparable to that in controls (Kemper and Bauman, 1998). However, it has to be noted that myelin staining used by the Loyesz method is not very sensitive and is unlikely to detect any abnormalities of myelin except if myelin was deficient. Thus, subtle quantitative abnormalities in myelination may still be responsible for our findings. Similarly, there is no evidence that axonal density is decreased in autistic brains even though white matter volume is increased in autism. Small neuronal size and increased cell packing density have been found in postmortem brain studies of autism (Casanova et al. 2002). These underlying abnormalities in cortical organization suggest that the white matter connected to these cortical structures may also be abnormally organized. Such an abnormal organization of white matter fibers result in decreased FA. Further reinforcing our findings, volumetric MRI studies in autistic subjects have found abnormal organization of frontal lobe white matter (Herbert et al. 2003, 2004) where there was excessive radiate white matter as compared with deep white matter.

Excessive Short Range Connectivity Hypothesis

Results from several studies have suggested that there may be excessive short range connectivity in autism (Casanova et al. 2002; Herbert et al. 2003, 2004). The observation of increased density of cortical minicolumns in autistic brains has been interpreted as suggestive of a skew in the ratio of short U fibers to long association fibers (Casanova et al. 2002). Similarly, the disproportionately increased radiate white matter observed by Herbert et al. (Herbert et al. 2003, 2004) supports this hypothesis because the radiate white matter contains predominantly short association fibers whereas deep white matter predominantly contains long tracts. Detailed studies investigating the relative position of short range and long range fibers in nonhuman primates have shown that the short and long association fibers are spatially localized to different territories in white matter (Petrides and Pandya 2007). The short fibers occupy the white matter just below the gray matter, whereas long fibers are located underneath the short fibers. Because partitioning white matter by volumetric MRI has limited capacity to resolve the short association fibers from long fibers, DTI tractography may be better suited to address the issue. Indeed, the topographic distribution of short and long range fibers is reproduced at a macroscopic level in the present study as seen in Figure 1 where the short range fibers are located just beneath the gray matter and the long range fibers occupy more central white matter regions.

In order to further test hypothesis of excessive short range connectivity, we examined the number and length of fibers delineated by the DTI studio software. It should be cautioned that the term "number of fibers" as used in current DTI analysis does not denote exact fiber count but, rather, some measure of fiber number. This "number of fibers" depends on FA and angular deflection thresholds and ROI placement (Thomas et al. 2005). Nevertheless, in the present the study, the FA and angular thresholds were always fixed and the ROIs were placed in accordance with the standardized protocols to derive the tracts (Mori et al. 2002). These protocols are now very well established (Mori et al. 2002, 2005; Wakana et al. 2004). At the level of major white matter tracts, the number of fibers as determined by tractography seems to be proportional to the

number of axons in the individual tracts. For example, the approximate descending order of number of axons (as estimated from postmortem studies) among the major cortico-cortical tracts is corpus callosum, superior longitudinal fasciculus, inferior longitudinal fasciculus, uncinate fasciculus, cingulum, fornix (Ashwell and Paxinos 2000) and this pattern of descending rank order is reproduced by the measure "numbers of fibers" as determined by tractography. Using this measure, we did not find evidence to support the excessive short range connectivity hypothesis, that is, we did not find increased numbers of short fibers in the autism group. Nevertheless, as the parameter "number of fibers" is relatively more variable than the parameters FA and ADC, the lack of difference between the 2 groups observed in the present study could reflect the imprecision in estimating the number of fibers. Thus, further advances in DTI methodology is still required to definitively resolve this issue. In the present study, DT-MRI was acquired in a diffusion scheme with only 6 noncollinear directions, but we repeated these 6 times in order to increase signal-to-noise ratio and reproduce water diffusion averaged from 6 directionalities. Highly myelinated fibers can be tracked with a model of 6 noncollinear directions and 6 measurements produced over 30 measurements (Jones et al. 1999) and signal-to-noise ratio over 20 (Huang et al. 2004). The new advances on MRI hardware and software allow the design of multicompartment model (voxels that contains white and gray matter) with higher diffusion directionalities (>35) to be able to perform probabilistic fiber tracking technique.

Even though the DTI tractography technique is relatively well established in isolating deep white matter tracts, its ability to isolate fiber tracts corresponding to specific cortical regions (e.g., individual Brodmann areas) is limited. In fact, the current study is a step in this direction by isolating the fiber tracts corresponding to a cortical region, but only at a lobar level. A recent DTI study parcellated prefrontal cortex into 8 sub-regions to investigate their connections to thalamus using probabilistic tractography (Behrens et al. 2003). However, even in this study the ROIs were placed in the subcortical white matter (rather than on the cortex itself), and the methodology does not lend itself to isolate either the short association fibers or all the fibers of the frontal lobe. Isolating the fibers from individual regions of the frontal lobe (e.g., orbitofrontal cortex, dorsolateral prefrontal cortex) may help in clarifying how the connectivity of these regions is important in autism and the inability to do so is a limitation of the current study. To overcome these issues, technological improvements in DTI and tractography software must 1st be developed.

An interesting finding in the present study is that the fiber length of the long association fibers (corresponding to the 2nd peak in Fig. 2) was higher in the ASD group than in controls. It is unclear from this study which specific long association tracts are involved. The predominant long association fibers belong to the anterior forceps of corpus callosum, inferior longitudinal fasciculus, superior longitudinal fasciculus, arcuate fasciculus, uncinate fasiculus and cingulum. Future tractographic studies investigating individual tracts may resolve this issue. Another issue is the difficulty encountered in tracking fibers at the regions where fibers cross. For example, the callosal fibers from the orbitofrontal and medial frontal regions can be easily isolated but the isolation of callosal fibers from lateral frontal region is usually not possible because these fibers need to cross through much stronger fibers of corona radiata. Further refinements in DTI technology may resolve some of these issues.

One of the mechanisms related to increased long range fiber length observed in this study could be altered serotonin synthesis during brain development as observed in our previous studies of autistic children using positron emission tomography (Chandana et al. 2005; Chugani et al. 1999). In fact, many previous studies have shown that serotonin acts as a neurotrophic factor involved in axonal outgrowth during development (Lauder 1990; van Kesteren and Spencer 2003). Indeed, serotonin depletion in neonatal cats resulted in increased numbers of callosally projecting neurons (Djavadian et al. 2003). By combining DTI with PET measures of serotonin synthesis, the role of altered serotonin in autism in white matter structural changes can be studied from both anatomical and functional perspectives.

Notes

Conflict of Interest: None declared.

Address correspondence to Senthil K. Sundaram, MD, PET Center, Children's Hospital of Michigan, 3901 Beaubien Blvd, Detroit, MI 48201, USA. Email: ssundaram@pet.wayne.edu.

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