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White matter fractional anisotrophy differences and correlates of diagnostic symptoms in autism

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Background: Individuals with autism have impairments in 3 domains: communication, social interaction and repetitive behaviours. Our previous work suggested early structural and connectivity abnormalities in prefrontal-striato-temporal-cerebellar networks but it is not clear how these are linked to diagnostic indices. **Method:** Children with autism (IQ > 70) aged 6 to 14 years old and matched typically developing controls were studied using diffusion tensor imaging. Voxel-based methods were used to compare fractional anisotrophy (FA) measures in each group and to correlate FA measures in the autism group with the diagnostic phenotype described by the Autism Diagnostic Interview – Revised (ADI-R) algorithm for ICD-10. **Results:** After controlling for the effects of age and white matter volume, we found that FA in the autism group was significantly lower than controls in bilateral prefrontal and temporal regions, especially in the right ventral temporal lobe adjacent to the fusiform gyrus. FA was greater in autism in the right inferior frontal gyrus and left occipital lobe. We observed a tight correlation between lower FA and higher ADI-R diagnostic algorithm scores across white matter tracts extending from these focal regions of group difference. Communication and social reciprocity impairments correlated with lower FA throughout fronto-striato-temporal pathways. Repetitive behaviours correlated with white matter indices in more posterior brain pathways, including splenium of the corpus callosum and cerebellum. **Conclusions:** Our data support the position that diagnostic symptoms of autism are associated with a core disruption of white matter development. **Keywords:** Magnetic resonance imaging, diffusion tensor, morphometry, brain, children.

Autism is a pervasive disorder of neurodevelopment which impairs social reciprocity and communication and causes repetitive movements and intense preoccupations. Optimal cognitive, socio-emotional, motor and language ability of typically developing children depends upon integration of complex interconnected brain systems (Paus et al., 1999). Therefore, it seems likely that the anatomy, connectivity and function of brain systems rather than discrete brain regions are affected by autism (McAlonan et al., 2005, 2002).

We previously identified grey matter abnormalities in a prefrontal-striato-parietal network in autism thought to be involved in information processing, social cognition and language (McAlonan et al., 2005, 2002). We observed significantly fewer and less positive inter-regional volumetric correlations in autism compared to controls, and interpreted this as evidence of widespread structural disconnectivity. In the first landmark study of connectivity in autism, Horwitz and colleagues (Horwitz, Rumsey, Grady, & Rapoport, 1988) reported significant differences in cortico-subcortical metabolic correlates in autism. Consistent with this concept are numerous reports of reduced functional 'connectivity' in autism (Castelli, Frith, Happé, & Frith, 2002; Just, Cherkassky, Keller, Kana, & Minshew, 2007; Just, Cherkassky, Keller, & Minshew, 2004; Koshino et al., 2005; Schultz et al., 2000). These assessments of connectivity are bolstered by recent findings that the basic microcolumnar organisational units of cortex are smaller and more tightly packed in frontal areas in autism, and this would ultimately lead to a marked alteration in neuronal projections (Casanova, Buxhoeveden, Switala, & Roy, 2002; Casanova et al., 2006).

Diffusion tensor imaging technology (DTI) can provide a proxy measure of microstructural properties of white matter. The degree of diffusion anisotropy of protons in tissue cells, represented by fractional anisotropy (FA) (Basser & Pierpaoli, 1996), is mediated by the organisation of the tissue, e.g., myelination, fibre size and density (Schmithorst, Wilke, Dardzinski, & Holland, 2002, 2005). Thus FA can be a useful indicator of white matter integrity. Among the first studies to use voxel-based methods to examine FA in autism, Barnea-Goraly reported lower FA in ventromedial prefrontal cortex, anterior cingulate, temporal lobe and corpus callosum of 7 male children (mean age 14.6) (Barnea-Goraly et al., 2004), while Keller, Kana, and Just (2007) found lower FA in corpus callosum and internal capsule of

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a mixed group of children and adults (age 10–35) (Keller et al., 2007). In contrast, very young children with autism (age 1.8–3.3) appear to have accelerated white matter maturation compared to controls (Ben Bashat et al., 2007). Thus, the white matter anomaly in autism remains unclear.

One reason for this may be that FA differences have to be sizeable and well localised to be easily detected in a group comparison study. Since autism exists along a spectrum, it is reasonable to assume that pathology in one individual may not exactly match another, and probing a distributed deficit is challenging. Moreover, a group difference approach carries with it an assumption that only areas of difference between controls and autism associate with autism-specific behaviours. In fact, it is entirely possible that more brain regions are involved in generating an autism phenotype than can be defined in a group difference approach. Therefore we suggest that a symptoms-led approach would complement the data available from group difference testing. The Autism Diagnostic Interview – Revised (ADI-R; Lord, Rutter, & Le Couteur, 1994) provides a comprehensive description of the triadic features of autism and generates a diagnostic algorithm. Although these categories are not independent (Constantino et al., 2004), they usefully describe a wide extent of symptoms and correlate with multiple cortical and sub-cortical grey matter volumes previously implicated in autism (Rojas et al., 2006) and consistent with the notion that autism is a system-level disorder.

We planned a voxel-based whole brain DTI study to examine white matter FA differences and phenotypic correlates in intellectually able children with autism. We hypothesised that children with autism would have significantly lower FA values in fronto-temporal 'social brain' systems compared to typically developing controls matched for age, gender, ethnicity and handedness. We expected to find negative correlations between white matter FA and ADI-R diagnostic algorithm scores, particularly in regions with significant group differences. Since ADI-R domains are not independent (Constantino et al., 2004) we did not rule out some overlap across FA correlates of each domain.

Methods

Subjects

Twenty-eight children aged 6–14 years old were recruited. Half were intellectually able un-medicated children with autism, and half were age-matched typically developing control children. Children with autism were recruited from a local child psychiatry clinic and their ICD-10 diagnosis of autism confirmed using the ADI-R (Lord et al., 1994, ©Western Psychological Services, 2003): Subscale A (Qualitative Abnormalities in Reciprocal Social Interaction), mean 19.1 (SD = 3.8); B (Qualitative Abnormalities in Communication) mean 14.9 (SD = 3.4); C (Restricted, Repetitive, and Stereotyped Patterns of Behavior) mean 6.1 (1.8). Controls were recruited from local schools and screened for major psychiatric illness using a parental Diagnostic Interview Schedule for Children for DSM-IV – Chinese version (Ho et al., 2005). We excluded children with a co-morbid psychiatric or medical condition (e.g., epilepsy); history of head injury; or genetic disorder associated with autism (e.g., tuberous sclerosis or fragile X syndrome). Every child's parent gave informed consent for the protocol approved by the hospital's Institutional Review Board, and each child gave his/her assent. Parents were compensated US$25 for time and expenses.

Data acquisition

Datasets were acquired on a GE Signa 1.5 Tesla system (General Electric, Milwaukee, WI, USA) as previously reported (Cheung et al., 2008): single-shot spin-echo echo-planar imaging with TR = 10,000 ms, TE = 100 ms, acquisition matrix = 128 × 128, and field of view = 28 cm, slice thickness of 5 with 1.5-mm gap. Diffusion-sensitising gradient encoding (Basser, Mattiello, & LeBihan, 1994; Basser & Pierpaoli, 1996) was applied in 25 directions using a diffusion-weighted factor b = 1200 s/mm^2. One image, B_0 was acquired without a diffusion gradient, b = 0 s/mm^2. In addition, dual-echo fast spin echo (PD/T2) data sets aligned to AC-PC across the whole brain were collected: 3 mm thick, TR 5–6 sec, TE 20/80 msec, Matrix 256 × 192.

Imaging processing and analysis

Image pre-processing. MRI datasets were preprocessed and analysed using SPM2 (Statistical Parametric Mapping 2, Wellcome Department of Cognitive Neurology, Institute of Neurology, UK) with the Diffusion II toolbox (http://sourceforge.net/projects/spmtools) running in MATLAB 6.5 (The MathWorks, Inc., Natick, Massachusetts, USA).

Diffusion tensor images were realigned to correct for motion. FA maps of each subject were calculated according to Basser and Pierpaoli's method (Basser & Pierpaoli, 1996). VBM preprocessing followed the optimised method (Good et al., 2001). Briefly, anatomical T2 and PD images of each subject were segmented using multi-spectral segmentation to enhance tissue classification accuracy (Styner, Charles, Park, Lieberman, & Gerig, 2002). The segmented tissue maps were linearly registered to the standard space and averaged to form a custom template. FA maps were spatially normalised (Ashburner & Friston, 2000) to the custom template by applying transformations derived from the normalisation of the white matter maps of the segmented B0 image. The normalised FA maps were smoothed with a 6mm full width half maximum kernel.

Before statistical analysis, an explicit mask defining the white matter of the group was created. The aim of this mask was to reduce type I error by restricting the voxel-based analysis to white matter only. This mask was created by averaging the white matter maps of all the subjects thresholded with the function (i1 > 0.2) in SPM2. Thus, only voxels that had more than 20% of white matter were included in the mask.

Statistical analysis

Group differences. The group difference analysis used the 'built-in' General Linear Model facility in SPM2. Confounding variables of age and white matter volume were included as 'nuisance variables' (covariate of no interest) in the design matrix. As described by Friston and colleagues (Friston et al., 1995) and used by others in similar study designs (Job et al., 2002), SPM2 allows a distinction between a covariate of interest (e.g., the ADI-R score in this study) and a potential confounder or so-called 'nuisance' variable (e.g., age or total white matter volume in this study). This enabled clusters of white matter with significant between-group differences in FA to be identified. Mean FA values of these regions were extracted and tested for Pearson correlation with ADI-A, B and C diagnostic algorithm scores using SPSS v15.0 (See electronic supplement for scatter plots.)

FA correlation with symptom domains. White matter FA correlates of ADI subscale scores were explored voxel-by-voxel across the whole brain in the autism group only, using standard linear regression in SPM2 with age and total white matter volume again defined as nuisance variables. This allowed mapping of additional white matter regions where FA was not necessarily significantly different from controls, but still implicated in the diagnostic phenotype.

Intracerebral voxels are not independent, making Bonferroni corrections inappropriate. We therefore adopted a 'seed and extension' approach which sets a high threshold for significance of a 'seed' voxel and then identifies adjacent voxels significant at a lower, but reasonable, level of significance to incorporate into the cluster (Alexopoulos et al., 2008; Baudewig, Dechent, Merboldt, & Frahm, 2003; Hoptman et al., 2004, in press; Lim et al., 2006). The thresholds here were selected with reference to two previous VBM studies on children with autism: (1) Barnea-Goraly et al. (2004) used height and extent of Z scores thresholded at $p < .05$ uncorrected; (2) Keller et al. (2007) thresholded their result at uncorrected $p < .0005$ with cluster extent of 10 voxels. The results here survived both levels of analysis with 'Seed' voxels at $p < 0.0005$ expanded to include voxels at $p < 0.005$ with a minimum voxel extent of 90. All MNI coordinates were converted to Talairach using a standard non-linear algorithm (Brett, Johnsrude, & Owen, 2002) for report below. Clusters were labelled according to the DTI-based white matter atlas from Johns Hopkins University (Hua et al., 2008; Mori, Wakana, Zijl, & Nagae-Poetscher, 2005; Wakana et al., 2007). In addition, although the groups were matched on IQ, we used multivariate general linear modelling in SPSS v15.0 to confirm that significant FA differences and correlations with ADI-R remained when IQ was co-varied.

Results

Subject characteristics

DTI data from 1 child with autism was rejected due to motion artefact. Therefore the 2 final groups comprised 13 children with autism (12 male and 1 female) and 14 controls (13 male and 1 female). There was no significant difference ($t = -0.653$, $p = 0.57$) in mean age of control group (9.9 years S.D. 2.5) and mean age of autism group (9.3 years S.D. 2.6). All subjects were right-handed and ethnic Chinese. IQ > 70 estimated using Raven's progressive matrices showed no significant group difference ($t = -1.557$, $p = 0.13$) (mean IQ of control group 111.9 S.D. 19.7; mean IQ of autism group 99.5 S.D. 21.9). ADI-R algorithm scores in the three domains of social impairment (A), communication impairment (B) and restrictive, repetitive and stereotypic behaviours (C) did not correlate with age or IQ in the autism group.

White matter FA group differences

White matter FA in bilateral prefrontal lobes (around BA10), right ventral temporal lobe, left middle temporal lobe and left cerebellar hemisphere was significantly lower in autism compared to controls (Table 1 and Figure 1). FA around the right superior longitudinal fasciculus and the left occipital lobe was significantly higher in autism.

Mean FA values of each of the abnormal clusters were extracted and their relationships with ADI-R domain scores are shown in Table 1. FA in frontal orbital and precentral clusters negatively correlated with ADI-A and ADI-B. Bilateral frontal and right ventral temporal clusters correlated negatively with ADI-A. Posterior parietal and occipital clusters correlated negatively with ADI-C. Thus, for those regions where group differences in white matter FA were significantly different in autism compared to control, we found the greatest difference in children with highest diagnostic symptom scores.

Voxel-wise symptom correlates

This set of analyses mapped regions of white matter, beyond those clusters with significant group difference from controls, where FA was correlated with ADI-R scores.

Voxel-wise FA correlates of ADI-A

ADI-A scores correlated negatively with FA in widespread white matter tracts throughout fronto-striatal-temporal regions in addition to the posterior corpus callosum (Table 2 and Figure 1). Negatively correlated clusters near the right ventral temporal region overlapped with the largest cluster of lower FA identified in the group comparison analysis above. There were no positive correlations between FA and ADI-A scores.

Voxel-wise FA correlates of ADI-B

Negative correlations between FA and ADI-B were in fronto-striatal areas and the posterior corpus

Table 1 Regions with FA differences in autism compared with controls

Suggested label and nearby Broadman area	Cluster size	Z*	Talairach co-ordinates X	Y	Z	ADI-A *r*	ADI-A 1-tailed	ADI-B *r*	ADI-B 1-tailed	ADI-C *r*	ADI-C 1-tailed
Regions with deficit in FA in children with autism.											
Frontal orbital cortex BA47, 11	194	4.16	−15	16	−18	−0.42	p ≤ 0.10	−0.66	p ≤ 0.01		
Precentral gyrus BA4, 6	172	4.02	25	−11	59	−0.46	p ≤ 0.05	−0.53	p ≤ 0.05		
Frontal pole BA11	232	3.47	−28	46	−7	−0.37	p ≤ 0.10				
Frontal pole BA11	177	3.67	21	45	−10	−0.38	p ≤ 0.10				
Fusiform gyrus BA19	357	3.79	36	−65	−8	−0.56	p ≤ 0.05				
Uncinate fasiciulus	170	3.44	12	14	−12					−0.54	p ≤ 0.05
Middle temporal gyrus BA 20, 21	522	3.42	−49	−22	−12					−0.33	p = 0.13
Regions with excess in FA in children with autism.											
Superior longitudinal fasciculus	280	3.74	37	−18	24						
Forceps major	387	3.51	−11	−77	28						

*'Seed' $p < 0.0005$, 'extension' $p < 0.005$, cluster size > 90 voxels. Coordinates are for the peak of each cluster. Labels do not necessarily define the full extent of each cluster; please see Figure 1. Pearson correlation coefficients for extracted mean FA values in each region and ADI-R symptom scores in the autism group.

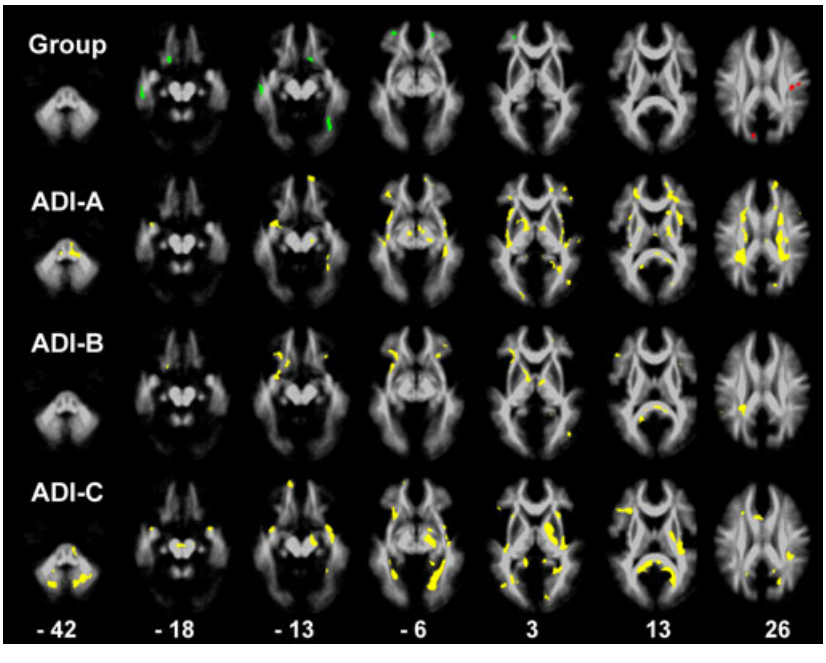

Figure 1 White matter FA group differences and voxel-wise correlates of diagnostic ADI-R scores. Top row: Group difference in FA between autism (n = 13) and controls (n = 14) (Green regions are lower FA in autism and red regions are higher FA in autism). Middle rows and bottom row: Voxel-wise FA correlates of ADI-A, ADI-B and ADI-C (Negatively correlated regions in yellow). All results threshold with 'seed' $p < 0.0005$, 'extension' $p < 0.005$, cluster size > 90 voxels

callosum. The correlation cluster in the left orbital frontal lobe overlapped with the cluster of significantly lower FA found in the group comparison analysis above. There were no positive correlations between FA and ADI-B scores (Table 3 and Figure 1).

Voxel-wise FA correlates of ADI-C

Clusters with negative correlation between ADI-C and FA were predominantly in the posterior regions, including white matter near basal ganglia, temporo-parietal lobe, splenium of the corpus callosum and cerebellum. There were only a limited number of negatively correlated clusters in anterior regions. One cluster in the left precentral gyrus showed a positive correlation between FA and ADI-C (Table 4 and Figure 1).

A final exploration of FA using multivariate general linear modelling in SPSSv15.0 indicated that further controlling for IQ did not change the significance of the group difference or ADI-R correlation result.

Table 2 Voxel-wise FA correlates of ADI-A (qualitative abnormalities in reciprocal social interaction)

			Talaraich co-ordinates		
Suggested label and nearby Brodman area	Cluster size	Z*	X	Y	Z
Frontal lobe					
Frontal forceps BA11	3169	4.72	12	55	−13
Superior frontal gyrus BA8	4117	4.92	−11	31	38
Medial frontal gyrus BA6	113	4.63	7	−18	48
Medial frontal gyrus BA6	689	3.52	12	−11	48
Inferior/middle frontal gyrus BA46	323	4.14	35	42	6
Middle frontal gyrus BA10	742	4.12	−23	41	10
Inferior/middle frontal gyrus BA47	417	4.11	−32	33	−3
Medial frontal gyrus BA6	97	3.99	−7	−20	50
Cingulate BA24, 31	908	3.94	−24	−17	40
Superior longitudinal fasciculus	133	3.61	−28	1	36
Superior frontal gyrus BA6, 8	171	3.58	19	10	55
Inferior frontal gyrus BA45	235	3.56	−42	28	4
Orbitofrontal fasiculus	656	3.54	−20	−13	24
Basal ganglia/Corpus callosum					
External capsule	1613	4.91	−35	−15	−1
External capsule	2383	4.07	−33	6	−4
Anterior limb of internal capsule	229	3.90	19	15	10
Posterior limb of internal capsule	474	3.79	19	−20	−2
Posterior limb of internal capsule	755	4.02	−8	−13	−4
Anterior thalamic radiation	121	3.76	3	−14	11
Genu of corpus callosum	95	3.59	−6	21	−4
Corpus callosum	266	4.01	4	−16	20
Temporal lobe					
Inferior longitudinal fasciculus	214	3.66	44	−73	6
Fusiform gyrus BA20,21	349	4.11	44	−2	−26
Fusiform BA19	143	3.38	34	−54	−8
Superior temporal gyrus BA22, 42	1419	3.59	53	−19	7
Parietal/Occipital/Brain stem/Cerebellum					
Superior longitudinal fasciculus BA 40	16912	5.88	37	−29	39
Superior longitudinal fasiculus	3317	5.00	−24	−35	26
Forceps major	213	3.76	−17	−84	10
Forceps major	388	3.76	17	−74	25
Forceps major	133	3.66	16	−94	12
Forceps major	2048	3.94	32	−56	6
Brain stem	1815	4.04	11	−39	−36
Cerebellum	1983	3.45	16	−64	−25

*'Seed' $p < 0.0005$, 'extension' $p < 0.005$, cluster size > 90 voxels. Coordinates are for the peak of each cluster. Labels do not necessarily define the full extent of each cluster; please see Figure 1.

Discussion

In this study we addressed the hypothesis that white matter maldevelopment associated with the autism phenotype. We found that children with autism have significantly lower white matter FA values associated with brain regions thought to be structurally and functionally abnormal in autism, i.e., prefrontal lobes and ventral temporal lobes. Lower FA within a majority of these regions correlated with more diagnostic symptoms scored using the ADI-R ICD-10 algorithm. However, a much more extensive white matter network was identified when we examined voxel-wise FA correlates of symptoms within 3 domains of the ADI-R algorithm.

There were two regions where FA values in the autism group were significantly greater than controls. These corresponded with the location of the superior longitudinal fasciculus in the right hemisphere and the left occipital lobe. The superior longitudinal fasciculus connects the right inferior parietal lobule and right inferior frontal gyrus which have been observed to be more active in autism during a working memory probe (Koshino et al., 2005). In the same study individuals with autism also showed greater activation in the left extrastriate cortex, near the cluster of higher FA in the occipital lobe observed here. The authors interpreted the right hemispheric findings as a possible consequence of the non-verbal strategies to process letter stimuli preferentially adopted by those with autism, while increased posterior activation suggested greater reliance on lower-order visual analysis (Koshino et al., 2005). It is feasible that our results represent some compensatory reorganisation of white matter in the same vicinities.

Areas with lower white matter FA in the present study are very close to grey matter regions previously observed to be smaller in autism including the right fusiform gyrus, superior temporal sulcus (McAlonan et al., 2005), and prefrontal regions (Abell et al., 1999;

Table 3 Voxel-wise FA correlates of ADI-B (qualitative abnormalities in communication)

			Talaraich co-ordinates		
Suggested label and nearby Brodman area	Cluster size	Z*	X	Y	Z
Frontal lobe					
Superior Frontal Gyrus BA8	227	4.17	15	28	40
Superior Frontal Gyrus BA6, 8	511	4.12	−18	1	55
Inferior Frontal Gyrus BA46	150	4.02	−47	27	12
Superior Frontal Gyrus BA8	129	3.94	−11	35	42
Middle/Superior frontal gyrus BA10	141	3.71	24	43	5
Frontal Pole BA47	118	3.55	37	37	−9
Superior longitudinal fasciculus	116	3.69	46	6	18
Superior longitudinal fasiculus	357	4.14	32	18	16
Uncinate fasciculus	252	3.86	31	24	−10
Uncinate fasiculus	212	3.66	−16	16	−13
Basal ganglia/Corpus callosum					
Internal capsule	475	4.28	6	−10	1
External capsule	2178	3.84	−32	7	−2
Internal capsule	368	3.69	−8	−4	4
Corpus callosum/Cingulum	220	3.53	−13	−27	30
Splenium of corpus callosum	1020	3.51	4	−34	19
Temporal/parietal lobe					
Superior longitudinal fasiculus/Inferior parietal lobe BA40	119	4.54	36	−31	38
Superior longitudinal fasiculus/Inferior parietal lobe BA40	400	4.21	−36	−50	42
Supramarginal Gyrus	351	3.92	−51	−21	31
Supramarginal Gyrus BA40	131	3.69	−50	−43	33
Inf longitudinal fasciculus/middle temporal gyrus BA39,19	251	4.12	42	−66	11
Cingulate gyrus BA32	374	3.64	−11	21	38
Cingulate BA23, 31	1028	3.85	−22	−36	28
Superior Temporal Gyrus BA21, 22	127	3.72	−50	−29	0
Fusiform gyrus BA20, 21	154	4.16	47	−2	−26
Forceps major	523	3.88	−18	−55	11

*'Seed' $p < 0.0005$, 'extension' $p < 0.005$, cluster size > 90 voxels. Coordinates are for the peak of each cluster. Labels do not necessarily define the full extent of each cluster; please see Figure 1.

McAlonan et al., 2005, 2002). Activity across this fronto-temporal network is thought to be desynchronised in autism (Castelli et al., 2002; Just et al., 2006, 2004; Kana, Keller, Cherkassky, Minshew, & Just, 2006; Koshino et al., 2005), therefore we speculate that the maldevelopment in white matter regions reported here contributes to structural disorganisation and neural dysfunction across these circuits.

Voxel-wise FA correlates with ADI-R scores identified more widespread white matter areas linked to diagnostic symptoms. The social and communication characteristics of autism assessed in ADI-A and ADI-B respectively were closely associated with fronto-striato-temporal white matter FA. More specifically these regions were around the medial prefrontal lobe, implicated in understanding of the mental state of others (Frith, 2001); around the right fusiform gyrus, needed for face recognition (Pierce & Courchesne, 2000; Pierce, Müller, Ambrose, Allen, & Courchesne, 2001); and near the superior temporal sulcus, involved in judging eye gaze, emotional expression and lip movement (Haxby, Hoffman, & Gobbini, 2000). In addition, FA measures in the uncinate fasiculus, connecting the basolateral amygdala with the frontal lobe, were related to ADI-B scores and to a lesser extent with ADI-C. This is particularly interesting given the central role the amygdala has been proposed to play in autism (Baron-Cohen et al., 2000; Grelotti et al., 2005; Munson et al., 2006). Anterior brain regions (amygdala and prefrontal cortex) are thought to provide a social signal to assist understanding of biologically significant movement or gesture (Allison, Puce, & McCarthy, 2000; Castelli et al., 2002). Our results suggest that white matter development in these high-order brain systems is closely associated with the communication impairment in autism.

All diagnostic domains of the ADI-R correlated with FA values in white matter around the basal ganglia. Taken together with previous reports of basal ganglia and thalamic grey matter volume abnormalities (McAlonan et al., 2005, 2002; Sears et al., 1999; Tsatsanis et al., 2003), and an absence of cortico-striatal volumetric correlations (McAlonan et al., 2005) in autism, the DTI evidence here suggests that the white matter microstructure within cortico-striatal systems has a critical role in autism. Each diagnostic domain negatively correlated with FA values within splenium of the corpus callosum. However, absolute FA scores in the corpus callosum of children with autism were not significantly different from controls in our study. In a comprehensive study of corpus callosum, Alexander et al. (2007) found that significant callosal DTI differences in autism were driven by a minority subgroup of participants with IQ measures at the lower end of

Table 4 Voxel-wise FA correlates with ADI-C (restricted, repetitive, and stereotyped patterns of behaviour)

			Talaraich co-ordinates		
Suggested label and nearby Brodman area	Cluster size	Z*	X	Y	Z
Negative FA correlations with ADI-C					
Frontal lobe					
Postcentral Gyrus BA4	138	5.14	38	−16	49
Corticospinal tract	280	4.00	19	−28	54
Corticospinal tract	747	3.59	19	−16	49
Corticospinal tract fron	863	3.81	−16	−7	43
Superior longitudinal fasciculus/Cingulate gyrus BA 32	416	4.00	−15	20	40
Middle Frontal Gyrus BA9, 46	103	3.99	−39	24	30
Medial/Superior frontal gyrus BA11	275	3.59	−15	55	−11
Basal ganglia/corpus callosum					
Retrolenticular part of internal capsule	535	3.53	−37	−28	9
Retrolenticular part of internal capsule	8993	4.60	33	−25	13
External capsule	1555	4.39	−26	14	−3
External capsule	462	3.81	27	20	4
Cingulum/ Corpus callosum	322	3.53	−7	16	24
Temporal/parietal lobe					
Uncinate fasciculus	297	3.69	−38	−2	−12
Superior Parietal Lobule BA7	387	3.70	27	−41	50
Superior longitudinal fasciculus	409	4.58	−28	−19	35
Superior longitudinal fasciculus/Cingulate gyrus BA31	161	4.45	−21	−37	40
Inferior longitudinal fasciculus	206	4.15	−42	−71	6
Inferior longitudinal fasciculus/Lingual gyrus BA19	10628	4.46	−27	−55	−1
Brain stem/Cerebellum					
Brain stem	804	3.61	9	−26	−35
Brain stem	429	4.24	−3	−22	−17
Cerebellum	763	4.59	−22	−70	−28
Cerebellum	5103	4.98	16	−68	−28
Positive FA correlation with ADI-C					
Superior longitudinal fasciculus/Precentral gyrus BA4	115	3.62	−42	−13	41

*'Seed' $p < 0.0005$, 'extension' $p < 0.005$, cluster size > 90 voxels. Coordinates are for the peak of each cluster. Labels do not necessarily define the full extent of each cluster; please see Figure 1.

normal. The corpus callosum is a critical inter-hemispheric connection important in establishing brain lateralisation with the splenium comprising pathways from occipital, temporal and parietal regions and it may be associated with the development of atypical cerebral lateralisation in autism (Escalante-Mead, Minshew, & Sweeney, 2003).

The negative correlation of ADI-C scores and white matter FA was most pronounced posteriorly, particularly in the cerebellum. Cerebellar involvement is among one of the most consistent findings in autism, with reports of cerebellar volume abnormalities (Courchesne, 1999; Courchesne et al., 1994; Courchesne, Yeung-Courchesne, Press, Hesselink, & Jernigan, 1988; Levitt et al., 1999) and post-mortem evidence of cerebellar malformation (Bailey et al., 1998; M. Bauman & Kemper, 1985; M. L. Bauman & Kemper, 2003). Our results agree that cerebellar abnormalities complicate autism but such pathology should be considered part of a more widespread brain disturbance. Other white matter correlates of ADI-C were located in motor regions, especially the basal ganglia and precentral areas. Interestingly, we found that one robust positive correlation between ADI-C and FA occurred in the precentral gryus. The study design does not allow us to interpret the causal direction of these changes. However, since the motor manifestations of autism incorporate additional, not fewer movements, it is tempting to speculate that 'over'-development of some motor pathways could be linked with the phenotype.

White matter FA and white matter volume in autism

It is difficult to reconcile observations of lower FA here with evidence of greater white matter volume in children with autism. In young children with autism pathological increases in head circumference appear suddenly between 2 and 14 months (Courchesne, 2002, 2004; Courchesne, Carper, & Akshoomoff, 2003). This excessive growth results in generally larger brains in children with autism up to the age of 12 years (Aylward, Minshew, Field, Sparks, & Singh, 2002; Herbert, 2005) and white matter volume changes primarily drive this size increase (Herbert et al., 2004). However, others have shown that, following very early hyperplasia, frontal lobe white matter volumes in autism actually increase at a much slower rate than in control children up to age 11 years (Carper, Moses, Tigue, & Courchesne, 2002). Magnetic resonance spectroscopy has recorded a generalised reduction in concentrations of a neuronal marker, n-acetylasparate (NAA), in the brains of children with autism (Friedman et al.,

2003) and no changes in the temporal and parietal lobe of adults (Murphy et al., 2002; Page et al., 2006), indicating that neurons are not numerically increased in autism. This makes it unlikely that any increases in white matter volume in early childhood are due to increased axonal connections. It is therefore possible that conventional white matter volumetric indices are rather non-specific, perhaps even reflecting non-neuronal proliferative processes such as activation and cell swelling of microglia and astroglia (Pardo, Vargas, & Zimmerman, 2005; Vargas, Nascimbene, Krishnan, Zimmerman, & Pardo, 2005). In our preliminary calculations (not presented here) the white matter volumes of children with autism in this study were not significantly greater than controls. Nevertheless, we included white matter volume as a covariable in our analysis to control for possible effects of this confounder.

Limitations

The link between white matter FA and autistic symptoms proposed here must be considered only a first step in exploring differences in connectivity using DTI. An important limitation of our work lies in adopting a correlational approach. The cause of any correlation may be indirect and is potentially unknown. It is not possible to determine whether the white matter changes we observed are due to developmental delay or more severe disruption of brain development. We did not include adults so we cannot comment on what happens to white matter organisation into adulthood. We are unable to determine whether the white matter changes observed are due to developmental delay or more severe disruption of brain development. Clearly further longitudinal studies are required. We restricted our study to intellectually able individuals with autism, so it is uncertain whether our findings can be applied to the entire autism spectrum. In addition, our numbers were modest and replication in larger samples will be necessary.

Conclusion

We believe our study provides important new insights into the role of white matter microstructural organisation in autism. White matter FA was significantly lower than typically developing controls in key regions of prefrontal lobe and right ventral temporal lobe. The correlational approach indicated that the autism phenotype was related to microstructural integrity of widespread developing white matter systems, with lower FA linked to higher diagnostic symptom scores. This may partly explain replication difficulties, as it implies that the sample characteristics in group comparison studies will affect the results. We believe the present findings support the growing conviction that autism is a disconnectivity disorder.

Supporting Information

Additional supporting information may be found in the online version of this article.

Appendix. Supplementary figures (Word document).

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

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Key points

- Previous work has suggested connectivity abnormalities in autism, but it is unclear how these are linked to diagnostic indices.
- In this study, FA maps of children with autism and matched controls were studied and correlated with phenotype described by ADI-R using voxel-based methods.
- We found autism to have lower FA in bilateral prefrontal and temporal regions, and higher FA in right inferior frontal gyrus and left occipital lobe.
- Tight correlation between lower FA and higher ADI-R scores were observed across tracts extending from focal regions of group difference.
- Our data support the position that diagnostic symptoms of autism are associated with a core disruption of white matter development.

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