

# White matter reduced streamline coherence in young men with autism and mental retardation

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**Background and purpose:** It has been proposed that white matter alterations might play a role in autistic disorders; however, published data are mainly limited to high-functioning autism. The goal of this study was to apply diffusion tensor imaging (DTI) and fiber tractography (FT) to study white matter in low-functioning autism and the relationship between white matter and cognitive impairment.

**Methods:** Ten low-functioning males with autism (mean age:  $19.7 \pm 2.83$  years) and 10 age-matched healthy males (mean age:  $19.9 \pm 2.64$  years) underwent DTI-MRI scanning. fractional anisotropy (FA) maps were analyzed with whole brain voxel-wise and tract-of-interest statistics. Using FT algorithms, white matter tracts connecting the orbitofrontal cortex (OFC) with other brain regions were identified and compared between the two groups. FA mean values of the autistic group were correlated with intelligence quotient (IQ) scores.

**Results:** Low-functioning autistic subjects showed a reduced tract volume and lower mean FA values in the left OFC network compared with controls. In the autistic group, lower FA values were associated with lower IQ scores.

**Conclusions:** We showed evidence of OFC white matter network abnormalities in low-functioning autistic individuals. Our results point to a relationship between the severity of the intellectual impairment and the extent of white matter alterations.

## Introduction

Autistic disorders are characterized by communication deficits, impairments in social interaction, stereotyped behaviors, and narrowed interests. In the Fourth Edition of the Diagnostic and Statistical Manual of Mental Disorders [1] autistic disorder is included amongst the pervasive developmental disorders (PDD).

Magnetic resonance imaging (MRI) has played an important role in suggesting abnormal functional brain organization in subjects with autism. In high-functioning autistic individuals, functional MRI

(fMRI) studies showed alterations of the functional anatomy of different cortical networks, both regarding the size and the areas included in the networks. These data, together with longitudinal studies showing altered white matter maturation curves [2,3] in PDD children, led to the development of the so-called underconnectivity theory of autism, which points to the interaction of multiple partial cortico-cortical and cortico-striatal disconnections as one of the main underlying pathological correlates of autistic disorders [4].

However, the vast majority of published imaging studies are limited to high-functioning autistic subjects [5], i.e., autistic individual individuals within the normal range of intellectual functioning. Little information is available about the functional brain architecture and white matter organization in low-functioning autistic patients who represent the

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majority of the autistic disorder population (approximately 76%) [6].

In this study, we used diffusion tensor imaging (DTI) and fiber tractography (FT) algorithms to explore the anatomical connectivity of the orbitofrontal cortex (OFC) in low-functioning autism. We decided to use the OFC as the main focus of our study as OFC is believed to be involved in different cognitive domains relevant to autism such as theory of mind and social cognition [7], as well as being implicated in stereotypes, one of the key features of autism [8]. Moreover, as the OFC is widely connected to other neural structures such as the anterior cingulate, prefrontal cortex and temporal pole [9], it could represent an informative model to study white matter alterations using FT-based technique. Lastly, OFC abnormalities have been reported both in anatomical [10] and functional imaging [11] studies of autism.

## Methods and materials

### Subjects

Subjects were 10 non-verbal, low-functioning young men with autistic disorder (mean age:  $19.7 \pm 2.83$  years, range: 18–27). The diagnosis of autism was based on DSM-IV-TR criteria [1]. All patients met ICD-10 criteria for autism and, before turning 18, had a score on the Childhood Autism Rating Scale [12] between 30 and 50 (mean score  $35.9 \pm 3.9$ ). Subjects with identifiable causes of autism and known neurological disorders, including epilepsy, were excluded. IQ was assessed using the Leiter International Performance Scale-Revised [13]. This scale provides a non-verbal measure of intellectual functioning. Mean non-verbal IQ of the autistic group was  $49.20 \pm 6.94$  (range: 38–58). Six subjects had an IQ score below 50, belonging, therefore, to the severe range of autism. All subjects were right-handed. After complete description of the study to the subjects, parents and/or guardians, written informed consent was obtained according to procedures approved by the local University Institutional Review Board. Autistic subjects underwent scanning under general anesthesia, whereas controls were scanned awake. Anesthesia, always with spontaneous breathing, was induced with intravenous propofol or inhalatory sevoforane. No complications occurred during or after MRI scanning. The control group included 10 healthy males and was matched with our autism group on age (mean age:  $19.9 \pm 2.64$  years, range: 18–26), socioeconomic status, and handedness. None had a personal history of neurological disorders or a family history of autism or mental retardation.

### Magnetic resonance imaging protocol

Scans were obtained on a 3T scanner system (Intera Achieva, Philips Medical Systems, Best, The Netherlands) equipped with both 80 mT/m/ms gradient coils and an 8-channel sensitivity encoding (*SENSE*) head coil. DTI was performed by using single-shot spin-echo echo-planar imaging (TR 10 000 ms; TE 59 ms; FLIP angle 90°; matrix size  $112 \times 112$ ; FOV 224 mm; slice thickness 2 mm; gap between slices 0; NSA 3; SENSE factor 2). We used a value of  $b = 1000 \text{ s/mm}^2$  and diffusion gradients applied in 33 non-collinear directions. DTI measures the physical restraints to the random motion of water molecules because of biological structures to probe white matter organization *in vivo*. Lower FA values are related to white matter damage or architectural disorganization [14].

### Tract-of-interest statistics

Diffusion tensor imaging data were processed by using the FMRIB's Diffusion Toolbox (FDT; <http://www.fmrib.ox.ac.uk/fsl/fdt/index.html>) software [15]. Pre-processing included correction for eddy currents distortions and motion artifacts; after these steps, FA parametric maps were obtained for each subject. Binary masks of the anatomical regions of interest (ROIs) corresponding to the left and right OFCs were identified using the MARINA software package (University of Giessen, Giessen, Germany). ROIs were used as starting areas for the probabilistic tractography fiber reconstruction algorithms [16]. Fiber tracking was initiated from all voxels within the seed masks to generate 11 000 streamline samples with a step length of 0.5 mm and a curvature threshold of 0.1. Of the 11 000 samples generated from each seed voxel, raw tracts were thresholded at least at 100 samples to remove voxels with very low connectivity probability [17].

Using the FLIRT toolbox of the FMRIB Software Library (FSL) library, a study-specific T2 template was created according to the iterative process described in Skelly *et al.* [18] and, then, used to normalize each subject's T2 image to standard space; the transformation matrix thus obtained was applied to the FA map and to the reconstructed white matter tracts. The obtained white matter tracts were then binarized and summed across subjects. Those results were used to generate a population probability map; to account for the probabilistic nature of FT and for inter-subject anatomical differences we retained in the population map only those reconstructed tracts, that were present in at least 40% of the subjects. Finally, left and right OFC network normalized volume and mean FA measures were calculated for each subject.

### Whole brain voxel-wise analysis of FA maps

All subjects' FA maps were realigned into a common space using the non-linear registration IRTK [19] and smoothed with a 4-mm full-width at half-maximum (FWHM) isotropic Gaussian kernel [20]. FA maps of the autistic and control groups were analyzed in SPM5 (<http://www.fil.ion.ucl.ac.uk/spm/>) using a voxel-wise approach. Because our *a priori* hypothesis was a specific alteration of OFC long-range connectivity as assessed by DTI-FT, we considered significant only clusters that were included inside the previously reconstructed white matter tracts, and survived both a threshold of  $P < 0.001$  uncorrected at the voxel level and of  $P < 0.05$  FDR-corrected for multiple comparisons at the voxel level over the reconstructed tracts masks. Clusters not included in the reconstructed white matter tracts were considered significant, if they survived a threshold of  $P < 0.05$  FDR-corrected for multiple comparisons at the voxel level over the whole brain. Note that all significance thresholds were at the voxel level and only clusters with at least 25 voxels are reported.

The Mann–Whitney *U*-test was used to compare the autism and control group in age, tract volumes, and mean FA within tracts. Spearman's rho correlation coefficients were used to examine associations between DTI measures and IQ scores. A statistical significance level of  $P < 0.05$  (two-tailed) was applied for all analyses. Results are given as means  $\pm$  standard deviations. *Post-hoc* power analysis was performed using NQUERY advisor (<http://www.statsol.ie>, version 6.0) for non-parametric data, fixing the alpha level at 0.01.

## Results

### DTI-metrics statistical analysis

The left OFC network' mean FA values were significantly lower in the autism group than in the control group ( $0.459 \pm 0.026$  vs.  $0.481 \pm 0.018$ ; degree of freedom [d.f.] = 18;  $P < 0.05$ ). No significant difference in right OFC network' mean FA was found (d.f. = 18;  $P = 0.123$ ), even though FA values were lower in the autism group than in the control group ( $0.452 \pm 0.023$  vs.  $0.468 \pm 0.018$ ). Moreover, the autism group presented a significantly lower mean left OFC network volume compared with controls ( $22\,523.7 \pm 2478.3$  vs.  $29\,327.2 \pm 1023.1$  mm<sup>3</sup>; d.f. = 18;  $P = 0.02$ ). No significant difference (d.f. = 18;  $P = 0.2$ ) in the right OFC network volume was found, even though it was lower in the autism group than in the control group ( $26\,938.4 \pm 3076.4$  vs.  $30,731 \pm 2124.1$  mm<sup>3</sup>). Statistical power was 69.98%.

Non-verbal IQ scores in the autism group correlated positively with the mean FA values of the left OFC network ( $r = 0.67$ ;  $P < 0.035$ ), and the correlation between IQ scores and mean FA values of the right OFC network was close to statistical significance ( $r = 0.57$ ;  $P = 0.08$ ). There was no significant correlation between IQ scores and left ( $P = 0.1$ ) or right OFC ( $P = 0.2$ ) network volumes.

### Spatial distribution and voxel-wise analysis of OFC anatomical connections

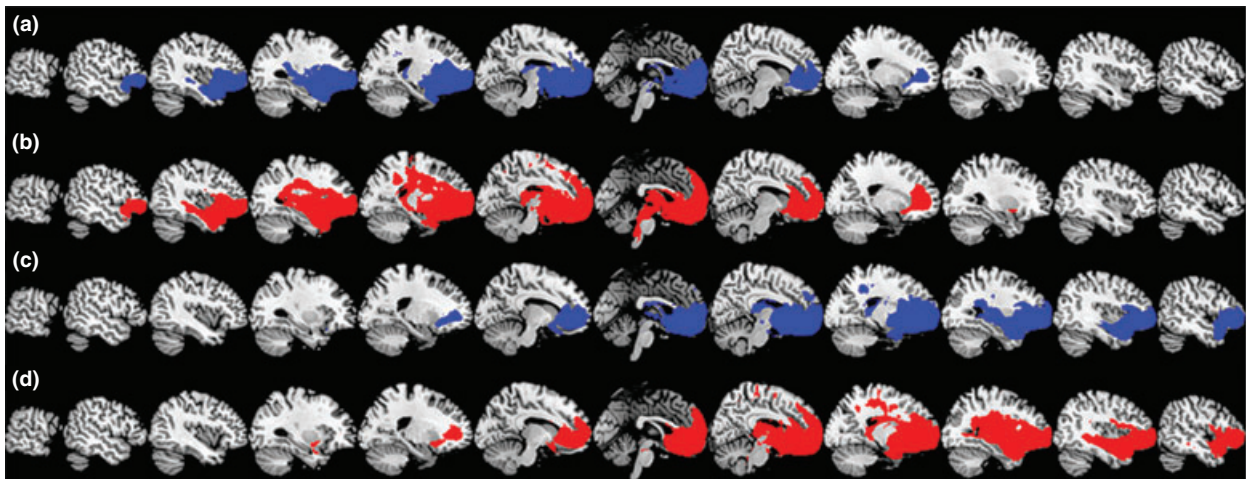
We evaluated the morphology of the OFC-related white matter tracts with the FT algorithms. Figure 1 shows the tracts, which we were able to trace consistently in at least 40% of the subjects of each group, and the direct comparison for patients' and controls' OFC connection patterns. The voxel-wise analysis revealed that the white matter abnormalities were significant in those white matter areas surrounding the cortical surface of the anterior cingulate and the inferior and medial frontal gyri bilaterally, and the right superior frontal gyrus (Fig. 2, Table 1).

Furthermore, some of the clusters identified in the FA maps voxel-wise analysis were located in the territory of the left uncinate fasciculus as represented in the FSL library white matter atlas.

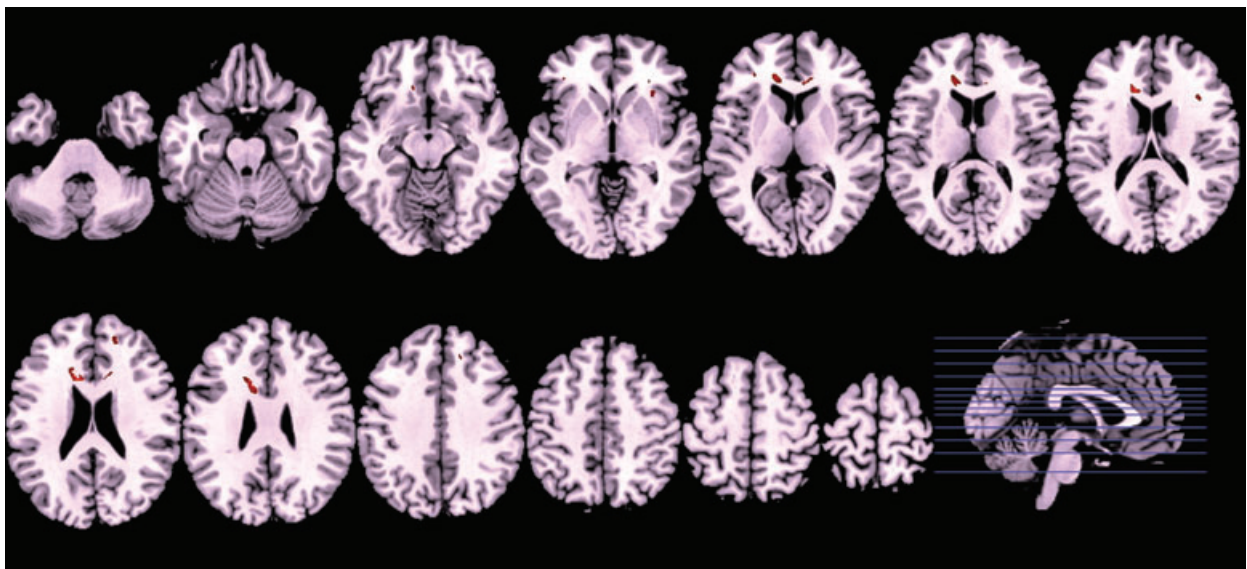
## Discussion

The goal of this study was to evaluate *in vivo* the anatomical connectivity of the OFC in adults with autism and mental retardation using DTI-derived metrics. The main findings of this study were an alteration of the structural properties (FA index) and of the spatial distribution of the white matter tracts connecting the OFC to other brain areas in autistic subjects. Our results seem to point to alterations of the structural integrity of the white matter connections of the OFC with different anatomical regions such as the cingulate gyrus, the temporal pole (through the uncinate fasciculus) and the prefrontal cortex (PFC).

Our voxel-wise analysis revealed an overlap between clusters of reduced FA and the left uncinate fasciculus in the autism group. This white matter bundle is a ventral limbic pathway that connects the temporal pole with orbital and medial frontal territories, and is involved in novel information processing, visual learning, and emotional self-regulation. Given the severe communication deficits of our patients, the involvement of the left and not of the right uncinate fasciculus may be related to a higher disease burden in the left hemisphere, compared with the right. However, we cannot exclude that we did not obtain significant statistical



**Figure 1** Panel A: autism group right orbitofrontal cortex (OFC) population connectivity map; Panel B: control group right OFC connectivity map; Panel C: autism group left OFC connectivity map; Panel D: control group left OFC connectivity map. Only reconstructed tracts present in at least 40% of subjects are shown.



**Figure 2** Anatomical overlay representation of the areas of reduced fractional anisotropy (FA) value in subjects with autism compared with controls according to voxel-wise analysis of FA maps. See methods section for details on statistical thresholds.

results for the right OFC white matter network because of the limited size of our sample and the small differences observed, which limited the power of our analysis. A recent study showed a reduction of left uncinate fasciculus mean FA values in children subjected to early socio-emotional deprivation [21]. Moreover, structural MRI data have shown a reduction of white matter concentration in the temporal pole region in autistic children [22].

Structural alterations of the uncinate fasciculus have also been found in schizophrenia [23]. Moreover,

schizophrenic patients present not only uncinate fasciculus alterations but also reduced FA in the anterior cingulate [24], a feature also observed in our population. Such common structural abnormalities might point to wider commonalities of these two disorders. More studies are needed to better explore these common findings.

We also showed that the structural properties of the OFC network, assessed with FA values, correlated with autistic subjects' non-verbal IQ. Subjects with lower IQ presented a more disrupted OFC white matter network

**Table 1** Voxel-wise comparisons of subjects with autism and controls fractional anisotropy (FA) maps

Cluster dimensions	<i>P</i> value		Cluster maxima (x, y, z)	Nearest gray matter area
	FDR-corrected	Voxel <i>z</i> scores		
981	0.006	7.32	-13, 15, 26	Left anterior cingulate
55	0.011	5.60	8, 54, -6	Right medial frontal gyrus
145	0.015	5.27	29, 32, 0	Right inferior frontal gyrus
51	0.017	5.14	18, 42, 32	Right superior frontal gyrus
153	0.019	5.08	-28, 31, -9	Left inferior frontal gyrus
51	0.025	4.82	-44, 19, -10	Left inferior frontal gyrus
133	0.030	4.66	8, 32, 9	Right anterior cingulate
171	0.034	4.54	-7, 25, -17	Left medial frontal gyrus
89	0.036	4.50	17, 48, 23	Right superior frontal gyrus
80	0.037	4.47	16, 34, 40	Right superior frontal gyrus
27	0.040	4.41	-17, 62, -6	Left medial frontal gyrus

Each cluster represent an area of reduced FA in the autistic group compared with controls. The coordinates are expressed according to the Montreal Neurological Institute template. See methods section for details on statistical thresholds.

than those with higher IQ. Moreover, compared with controls, autistic subjects presented a higher inter-subject variation of OFC network FA indices, as shown by the large standard deviations of autistic group FA values. It would be interesting to expand our findings also to other white matter networks; a recent study in patients with Asperger's syndrome, for example, found reduced FA values in deep cerebellar white matter and in the superior cerebellar peduncles [25]. Studies with more statistical power are warranted to clarify this issue.

There are some differences between our results and other studies of white matter organization in PDD. A recent study [26], showed an increase in FA values in the left hemisphere white matter in autistic children whilst our data show a decrease in mean FA. These divergences might be because of differences in the studied populations (children versus adults), to different experimental paradigms as well as the limited statistical power of our study and of the majority of the published ones, because of small samples and small differences observed. However, as all autistic subjects in our study presented with mental retardation, one possible interpretation of our findings could also be the existence of qualitative anatomical differences between autistic people with and without mental retardation. Future studies comparing autistic subjects with and without mental retardation, as well as with non-autistic subjects with mental retardation could help to better understand whether the white matter abnormalities shown in our study are more related to mental retardation, to autism or to both.

Our results also bear upon the controversy surrounding hemispheric asymmetries in autism. Whilst we found more severe white matter alterations in the left hemisphere, the published studies of white matter structural alterations, limited to high functioning verbal autistic subjects, revealed more severe alterations in the right hemisphere [20,27].

One factor that could explain this difference is that we enrolled only autistic subjects with a severe verbal impairment. Whilst communicative deficits are one of the core features of PDD, their pathophysiology is still obscure. Further studies, comparing autistic subjects with different severity of communicative deficits are needed to better clarify these results.

The major limitation of this research is the small number of subjects enrolled and, therefore, its limited statistical power. However, the study of low-functioning autistic subjects, poses several challenges to the experimenters. MRI-DTI studies can be particularly challenging, as they necessitate that the participants lie still in the scanner for a number of minutes or, if a subject is unable to do so, s/he has to undergo general anesthesia. Anesthesia might be in itself a confounding factor when comparing anesthetized subjects with non-anesthetized controls; however, it is difficult to exclude such a factor in a MRI study of low-functioning autism. Lastly, it must also be pointed out that DTI, like all echo-planar imaging-based techniques, is sensitive to magnetic susceptibility and geometric distortion artifacts, especially in those brain regions near to the skull base; high-field magnets and of dedicated imaging techniques, however, can be used to reduce the impact of these artifacts on DTI. Nevertheless these technical limitations as well as the surrogate nature of MRI quantitative markers such as FA indices must be taken in account to correctly interpret DTI data.

In conclusion, we believe that DTI techniques and their future advances may help to broaden our knowledge of autistic disorders.

## Financial disclosures

The authors report no conflict of interest.

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