

***Fiber tract associated with autistic traits in healthy adults***

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**Abstract**

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder with impairment of social communication and restricted and repetitive behaviors. Reduced fractional anisotropy (FA), a measure of white matter integrity, in the posterior superior temporal sulcus (pSTS) is related to ASD. However, there are several major fibers in pSTS, and it is unknown which of them is associated with ASD. We investigated FA in correlation with autistic traits assessed by autism spectrum quotient (AQ) in 91 healthy adults using tract-based spatial statistics (TBSS). Then, of the fibers in pSTS, we identified the one in which FA was linked to the AQ score using tractography. TBSS revealed that AQ was correlated with FA of white matter in several regions such as the frontal lobe, parietal lobe, occipital lobe and temporal lobe including pSTS. With further analysis using tractography, we confirmed that FA alteration in pSTS was located on the inferior fronto-occipital fasciculus (IFOF). IFOF has a critical role in processing socio-emotional information. Our findings suggest that of the fibers in pSTS, IFOF is a key fiber that links to autistic traits in healthy adults.

**Highlights**

TBSS revealed that autistic traits are associated with white matter integrity.

Tractography identified the fiber tract in which FA was linked to autistic traits.

The fiber tract in pSTS region was the inferior fronto-occipital fasciculus.

### **Keywords**

Autism, tract-based spatial statistics, tractography, posterior superior temporal sulcus, inferior fronto-occipital fasciculus

**Introduction**

Autism Spectrum Disorder (ASD) was proposed as a developmental disorder with a triad of impairments in social interaction, communication, and imagination (Wing, 1988, Wing, 1997), and it was defined in the Diagnostic and Statistical Manual of Mental Disorders fifth edition (DSM-5) as neurodevelopmental disorder with impairment of social communication and restricted and repetitive behaviors (American Psychiatric Association, 2013). ASD was previously considered to have aberrant brain function in specific regions such as the cerebral cortex, cerebellum, amygdala, basal ganglia, and mesolimbic system (Allely et al., 2014, Cauda et al., 2011, Courchesne, 1997, Stanfield et al., 2008, Via et al., 2011). Recently, ASD has been increasingly considered a disorder of impaired brain networks (Just et al., 2012, Kana et al., 2011, Vissers et al., 2012), in which white matter abnormalities can be the neural underpinnings. Previous diffusion tensor imaging (DTI) studies in people with ASD repeatedly reported the reduction of fractional anisotropy (FA), which indicates impaired white matter integrity (Walker et al., 2012). A recent study has examined the developmental trajectory of white matter in 6- to 24-month infants with high risk of ASD, showing rapid increase and subsequent stoppage of FA of several fiber tracts in very young infants with ASD and supporting the consistent findings of reduced FA in individuals with ASD (Wolff et al., 2012). Brain alterations in individuals in ASD are also evident in high autistic traits in healthy people (Nummenmaa et al., 2012, Wallace et al., 2012), which is in line with the notion that ASD forms a continuum from autism to healthy population (Baron-Cohen et al., 2001a, Constantino and Todd, 2003, Robinson et al., 2011). These findings suggest that there is reduced white matter integrity

in the brain of people with ASD and that such alterations can be related to autistic traits in healthy people.

The posterior superior temporal sulcus (pSTS), which has many inputs and outputs of white matter fibers (Lahnakoski et al., 2012), has been repeatedly reported to play a key role in the pathophysiology of ASD (Pelphrey et al., 2011, Zilbovicius et al., 2006). In addition, growing evidence has indicated that there is reduced FA of white matter around pSTS of people with ASD. Reduced FA in the white matter region adjacent to pSTS has been reported by several DTI studies using voxel-based morphometry (VBM) (Barnea-Goraly et al., 2004, Bloemen et al., 2010, Groen et al., 2011), a widely used automated imaging-analysis method for exploring regional brain alterations. Recently, some studies used tract-based spatial statistics (TBSS), which is a voxelwise analysis method for DTI and is robust for registration errors inherent in VBM (Smith et al., 2006), to show a reduction of FA in the pSTS region in children and adolescent with ASD (Barnea-Goraly et al., 2010, Jou et al., 2011b, Shukla et al., 2011a). However, it is difficult from these voxelwise techniques to know on which fiber tracts these FA changes are located, because several white matter tracts run adjacent to pSTS, including the arcuate fasciculus (AF), inferior longitudinal fasciculus (ILF) and inferior fronto-occipital fasciculus (IFOF) (Catani and de Schotten, 2012).

How can we investigate the detailed location of FA change? Diffusion tractography reconstructs each individual white matter fiber trajectory using directional information in DTI data. Integrating tractography technique with voxelwise method is helpful for investigating on which fiber tracts FA is reduced in the pSTS-adjacent white matter region. So far, two studies have employed this strategy. Kumar

et al found reduced FA around the STS region in TBSS in young children with ASD and confirmed the reduction of FA for several fibers using tractography, although their findings were not significant after correcting for IQ (Kumar et al., 2010). Using VBM, Jou et al showed clusters of FA reduction in the pSTS region in children with ASD, and then revealed by tractography that clusters can be either on IFOF or ILF (Jou et al., 2011a). However, it still remains unclear which fibers in pSTS (e.g., ILF and IFOF) have FA alterations in people with ASD.

In the current study, we aimed to identify the anatomy of autistic trait-related FA alterations in the pSTS region with a method using TBSS and subsequent tractography. We used the autism spectrum quotient (AQ), a well-validated self-report questionnaire of autistic tendency (Baron-Cohen et al., 2001b, Wakabayashi et al., 2006), for a large sample of healthy adults and investigated regions where FA values would be associated with autistic traits using TBSS. We then performed tractography and examined in which fiber tract in pSTS such autistic-trait-related FA alterations were located.

## **Materials and Methods**

### **Participants**

Ninety-seven healthy adults participated in this study (mean age 28 years; age range, 19-55 years; 47 females; 5 left-handed subjects). Autism spectrum traits were measured with AQ for all participants. To assess the intelligence quotient (IQ), vocabulary task and block design of the Wechsler adult

intelligence scale revised (WAIS-R) were carried out. The structured clinical interview for DSM-IV axis I disorders (SCID) was performed by trained psychiatrists to check for current or past history of psychiatric disorders. Two participants were excluded due to a history of depression and possible onset of obsessive-compulsive disorder. Subjects underwent brain MRI according to the protocol described below. MRI could not be performed for 1 subject due to cosmetics, and DTI images could not be used for analysis of 3 subjects due to head motion. Finally, 91 participants were included in the analyses. The research protocol was approved by the institutional review board and the ethics committee of Kyoto University Graduate School of Medicine. Written informed consent was obtained from all participants at the time of their visit.

#### Autism spectrum quotient (AQ)

We adopted the Japanese version of the AQ scale (Wakabayashi et al., 2006) to measure the autistic tendencies of individuals. This scale is a self-administered questionnaire originally developed to measure the degree of autistic traits of individuals with normal intelligence (Baron-Cohen et al., 2001b). This questionnaire consists of 50 questions. Each question is rated on four choices: definitely agree (1), slightly agree (2), slightly disagree (3), and definitely disagree (4). When the participant recorded abnormal or autistic-like behavior as either definitely or slightly agree/disagree, 1 point was scored for the item. We used only total scores for the analysis. Higher scores indicate higher autistic tendency.

## MRI acquisition

DTI data were acquired using single-shot spin-echo echoplanar sequences and structural MRI data with 3-dimensional magnetization-prepared rapid gradient echo (3DMPRAGE) sequences, on a 3.0-T MRI unit (Trio; Siemens, Erlangen, Germany) with a 40-mT/m gradient and a receiver-only eight-channel phased-array head coil. The parameters for diffusion-weighted data were as follows: TE = 96 ms, TR = 10,500 ms,  $96 \times 96$  matrices, FOV =  $192 \times 192$  mm, 70 contiguous axial slices of 2.0 mm thickness, 81 non-collinear axis motion probing gradient,  $b = 1,500 \text{ s/mm}^2$ . The  $b = 0$  images were scanned preceding every nine diffusion-weighted images, thus consisting of 90 volumes in total. Parameters for the 3D-MPRAGE imaging were as follows: TE = 4.38 ms; TR = 2000 ms, inversion time = 990 ms,  $240 \times 256$  matrices, FOV =  $225 \times 240$  mm, resolution =  $0.9375 \times 0.9375 \times 1.0$  mm, and 208 total axial sections without intersection gaps.

## Data preprocessing

All DTI data processing was performed using programs in the FMRIB Software Library (FSL) version 4.1.9 (<http://www.fmrib.ox.ac.uk/fsl>). Source data were corrected for eddy currents and head motion by registering all data to the first  $b = 0$  image, with affine transformation. The FA maps were calculated using the DTIFIT program implemented in FSL. For tractography, probability distributions on 2 fiber directions were modeled at each voxel using FSL's BedpostX program (Behrens et al., 2007), based on a multifiber diffusion model. The 3D-MPRAGE images were preprocessed using the FreeSurfer

software package version 5.0.0 (<http://surfer.nmr.mgh.harvard.edu>). In brief, the processing stream included a Talairach transformation of each subject's native brain, removal of nonbrain tissue, volumetric subcortical labeling, and surface-based segmentation of gray matter (GM)/white matter (WM) tissue. In the automatic segmentation procedure, each voxel in the normalized brain volume was assigned a label based on an atlas containing probabilistic information about the location of structures, such as the thalamus, caudate, pallidum, putamen, accumbens area, hippocampus, amygdala, cerebral white matter, cerebral cortex, brain stem, and non-brain regions. The cerebral cortex was parcellated into gyrus-based regions of interest, which were used as seeds and targets for the following tractography.

#### Voxelwise DTI data analysis

TBSS version 1.2 was used for voxelwise statistical analysis. First, FA data of all subjects were normalized into a common space using the nonlinear registration tool FNIRT; normalized FA images were averaged to create a mean FA image, which was then thinned to create a skeleton taking only the centers of white matter tracts. This skeleton was thresholded at FA of 0.2. Voxel values of each subject's normalized FA map were projected onto the skeleton by searching the local maxima along the perpendicular direction from the skeleton. Voxelwise permutation-based nonparametric inference (Nichols and Holmes, 2002) was performed on this skeletonized FA data using FSL Randomize ver. 2.9. We performed multiple regression analysis with AQ as covariate of interest and age and gender as nuisance covariates. Both positive and negative correlations with AQ were tested, with 10,000

permutations. The statistical threshold was set at  $P < 0.05$ . Multiple comparisons were corrected using threshold-free cluster enhancement (TFCE) (Smith and Nichols, 2009). TFCE does not need an arbitrary clusterforming threshold while preserving the sensitivity benefits of clusterwise correction. We identified the location of the clusters using the Johns Hopkins University DTI-based White Matter Atlas (<http://cmrm.med.jhmi.edu>) and checked potential fiber tracts that may include the clusters as follows.

### Tractography

We used FSL's ProbtrackX program for depicting those fiber tracts. The seeds and targets of tractography were selected from cortical parcellation created by FreeSurfer (Figure 1A). Exclusion masks were also created from FreeSurfer parcellation to exclude anatomically invalid fibers. These masks were transformed from each subject's 3D-MPRAGE space to the diffusion space by applying the rigid-body transformation matrix, which was calculated by FSL's FLIRT program. Probabilistic tractography was performed in the diffusion space (Figure 1B), and streamline samples were traced through the probabilistic distributions of fiber direction, with 5,000 iterations per seed voxel (curvature thresholds = 0.5). Each tract was created in the 3D-MPRAGE space (Figure 1C) and thresholded to exclude voxels in which the streamline sample count corresponded to the lower 25% of the outer-tail of the histogram, to eliminate extraneous tracking results. The thresholded tracts were transformed back into diffusion spaces (Figure 1D) and overlaid on the significant clusters of TBSS that were transformed into individual diffusion spaces (Figure 1E,F).

### Identification of tracts

We tracked AF, ILF and IFOF. For AF, we chose the banks of the superior temporal sulcus as seed and the middle frontal gyrus as target for tractography from parcellation of FreeSurfer. ILF was traced from the temporal lobe to the extrastriatal region. For this analysis, we used the temporal pole, superior temporal gyrus, middle temporal gyrus, inferior temporal gyrus and fusiform gyrus as temporal lobe from parcellation of FreeSurfer. We also used the lateral occipital gyrus, cuneus and lingual gyrus as extrastriatal regions in a similar way. Another tractography isolated IFOF by tracking from the orbitofrontal area to the occipital lobe. We used lateral orbitofrontal, medial orbitofrontal and parsorbitalis as the orbitofrontal area and lateral occipital, cuneus, pericalcarine and lingual gyrus as the occipital area in a similar way.

### Investigation of TBSS cluster

We tried to identify the fiber tracts upon which the significant cluster shown in the TBSS analysis was located. For this purpose we calculated the extent of overlap between the cluster and each fiber tract. Here we call each cluster transformed in diffusion space as the Cluster of Interest (Figure 1E,H), and the overlap between a fiber tract and the Cluster of Interest as the Overlap (Figure 1G,I). We calculated the Rate of Overlap as the volume of the Overlap divided by that of the Cluster of Interest.

## Results

The demographic data and AQ score are shown in Table.1. There was no gender difference in age and AQ score. AQ was correlated with age. Although two participants had higher AQ score than the cut-off level, we confirmed that they revealed no impairments in social interaction and communication in a detailed interview by experienced psychiatrists.

TBSS revealed 5 clusters with a significantly negative correlation between FA and AQ (Table 2). No clusters showed a significantly positive correlation. We did not find any significant correlation between AQ and any of the other DTI parameters, such as mean, axial and radial diffusivity. According to the JHU white matter atlas, the largest cluster (shown in light blue in Figure 2) appeared to fornix (Fx), inferior longitudinal fasciculus (ILF), inferior fronto-occipital fasciculus (IFOF), posterior limb of internal capsule (PLIC) and corona radiata. The second cluster (red in Figure 2) included the corona radiata and corpus callosum (CC). The third cluster (green) included AF. The fourth (yellow) and fifth (purple) clusters included the anterior thalamic radiation (Figure 2). The average FA value of each cluster for each subject was extracted to confirm the correlations between FA and AQ score. The correlation coefficients were significant with  $r = -.402, -.432, -.395, -.338,$  and  $-.285$  (each  $P < .05$ ), respectively. We also conducted TBSS analysis with each subscale of AQ. We found no significant correlation between FA and each subscale.

Next, we tried to identify the fiber tract upon which the cluster adjacent to pSTS was located. The

largest cluster covered the white matter near pSTS. When shown in 3-dimensional (3D) view, it was found that it could be divided into 4 subdivisions: 1) posterior limb of internal capsule and corona radiata, 2) fornix part, 3) occipital part, and 4) white matter close to pSTS (Figure 3). We picked the subcluster adjacent to pSTS out from the mean FA image (standard space) manually and found that it had 413 voxels and occupied the temporal stem from  $y = -18$  to  $-41$  and from  $x = -28$  to  $-41$  in Montreal Neurological Institute coordinates. We transformed it into individual diffusion space and calculated the Rate of Overlap in each of the fibers of IFOF, ILF and AF. The Rates of Overlap were  $0.70 (\pm 0.16)$  for the IFOF,  $0.27 (\pm 0.17)$  for ILF and  $0.04 (\pm 0.06)$  for AF (Figure 4).

## Discussion

We found that autistic traits in healthy adults were significantly correlated with the FA values of white matter in several regions such as IFOF/ILF, PLIC/corona radiata, fornix, corpus callosum (CC), AF and anterior thalamic radiation (ATR). In the region of pSTS, a key brain area in the pathophysiology of ASD, we found altered white matter integrity in IFOF.

The fibers in which the current TBSS revealed FA negatively correlated with AQ were similar to those where previous TBSS was found with reduced FA in ASD compared with controls (Jou et al., 2011b, Shukla et al., 2011a). The locations of clusters were similar to those of previous studies reporting IFOF/ILF, CC, AF and ATR. It was expected that no fiber showed a positive correlation in FA value with AQ (Shukla et al., 2011b, Vissers et al., 2012). Similar white matter regions were, though to a lesser

extent, also reported to be aberrant in unaffected siblings of children with ASD (Barnea-Goraly et al., 2010). Thus, combined with these previous studies, our results indicate the existence of neural underpinnings of autistic traits, which have ASD at one end and autistic tendency among healthy at on the other.

These fiber tracts are known to be related to cognitive features of ASD. CC, for example, is related to cognitive and social function (Paul et al., 2007). In addition, people with autism have altered white matter integrity in CC (Alexander et al., 2007, Thomas et al., 2011). Our finding of the correlation between FA value in CC and AQ are in line with these previous findings. We also found a negative correlation between integrity in AF and AQ. AF is an important fiber for phonological processing (Duffau, 2012, Yeatman et al., 2011), which is impaired in children with ASD and their relatives (Wilson et al., 2013). Our findings in this fiber were consistent with previous studies that showed altered integrity of AF in ASD (Fletcher et al., 2010). Furthermore, we found a significant negative correlation between AQ and FA in anterior thalamic radiation (ATR). Similar patterns of reduced FA of ATR were found in boys with ASD (Cheon et al., 2011).

Analysis using tractography identified that the area with a negative correlation to AQ score was in IFOF adjacent to the pSTS region. This area was detected as the cluster that seemed to continue from AF. However, after confirming the fiber orientation of each individual with actual tractography, it was clear that the parietal part and temporal part of AF were not present simultaneously in a sagittal plane because AF runs backward from the frontal lobe and changes its course downward as well as laterally (Figure 4).

According to the atlas, the cluster in pSTS appeared to be on IFOF or ILF, but it was difficult to specify the individual fibers. By using tractography, we were able to determine the cluster on IFOF but not on ILF.

IFOF seemed to be an important fiber for connecting the frontal lobe with other lobes, although some researchers have insisted that IFOF did not exist (Schmahmann and Pandya, 2007). They claimed that the associated fiber, which we call IFOF, did not exist in macaque monkey and that the fiber in the external capsule was a projection fiber. However, researchers of postmortem human brain reported that there is a fiber that runs from the frontal lobe to the occipital lobe via the external capsule, calling it IFOF (Martino et al., 2010). DTI studies have also shown IFOF repeatedly (Catani et al., 2002, Wakana et al., 2004), suggesting that it was peculiar to human beings (Thiebaut de Schotten et al., 2012). Research on the function of IFOF with intraoperative electrical stimulation has revealed its role in semantic processing (Duffau et al., 2005). A study of brain injury also showed that IFOF was related to semantic processing (Han et al., 2013), which has been reported to be impaired in people with ASD (Boucher, 2012). Furthermore, a study of brain injury indicated that IFOF was associated with perception of facial expression (Philippi et al., 2009). The processing of facial expression is aberrant in people with ASD (Losh et al., 2009, Weng et al., 2011). Thus, variation of fiber integrity of IFOF might lead to autistic features, as the fiber plays a key role in processing social information.

We experienced several limitations in this study. First of all, we did not investigate the brains of individuals with ASD. Further study needs to include both subjects with and without ASD. On the other

hand, some subjects with ASD have mental retardation, which might affect the results. The current study can remove this confounding factor because all of our subjects had normal IQ. Second, we could not distinguish IFOF from extreme capsule (EmC). The part of EmC, not IFOF, might have an alteration of FA associated with AQ. Finally, it was not revealed which cortices the altered white matter actually connects, although we found that the pSTS parts of IFOF have reductions of FA values.

In conclusion, we showed that typically developed individuals with high autistic traits tend to show lower FA in several brain areas including pSTS. Furthermore, by combining TBSS and tractography, we were able to reveal that the white matter associated with autistic trait was mainly on IFOF in the pSTS area. Our findings suggest that reduced white matter integrity in pSTS in ASD might stem from disruption of IFOF. This result from healthy adults could contribute to a better understanding of the whole pathological mechanism underlying ASD.

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## **Contributors**

Hidehiko Takahashi and Toshiya Murai designed the study. Genichi Sugihara wrote the protocol.

Manabu Kubota and Akihiko Sasamoto operated the magnetic resonance imaging (MRI) machine.

Toshihiko Aso and Hidenao Fukuyama supervised the operation of MRI and analysis of the data. Jun

Miyata managed the analyses. Kimito Hirose wrote the draft of the manuscript. All authors contributed

and have approved the final manuscript.

## **Conflict of interest**

All authors declare that they have no conflicts of interest.

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Table 1

## Demographic data

|        | Average±SD   | Range  | Sex difference | Pearson Correlation |           |
|--------|--------------|--------|----------------|---------------------|-----------|
| AQ     | 17.00±7.04   | 5-34   |                |                     |           |
| male   | 18.32±7.56   |        | n.s.           |                     |           |
| female | 15.59±6.05   |        |                |                     |           |
| Age    | 28.21±10.92  | 19-55  |                | With AQ             |           |
| male   | 27.87±9.31   |        | n.s.           | r = -.293           |           |
| female | 28.57±12.29  |        |                | (p < .01)           |           |
| IQ     | 116.26±13.57 | 80-145 |                | With AQ             | With Age  |
| male   | 121.33±11.49 |        |                | r = .097            | r = .252  |
| female | 110.85±13.64 |        | p < .001       | (n.s.)              | (p < .05) |

Distribution of AQ is similar to that expected. SD, standard deviation; AQ, autism spectrum quotient; IQ, intelligence quotient; n.s., not significant

Table 2

## Results of TBSS

| Area or possible tract fibers         | voxels | t value | Z MAX |     |    |
|---------------------------------------|--------|---------|-------|-----|----|
|                                       |        |         | x     | y   | z  |
| 1) PLIC/corona radiata, IFOF, ILF, Fx | 1708   | 4.02    | -40   | -44 | -3 |
| 2) corona radiata, CC                 | 373    | 5.01    | -17   | -36 | 28 |
| 3) arcuate fasciculus                 | 128    | 4       | -35   | -23 | 26 |
| 4) anterior thalamic radiation        | 69     | 2.86    | -25   | 27  | 11 |
| 5) anterior thalamic radiation        | 45     | 3.03    | -21   | 23  | -1 |

5 clusters were found to be negatively correlated with AQ. TBSS, tract-based spatial statistics; MAX, maximum; PLIC, posterior limb of internal capsule; IFOF, inferior fronto-occipital fasciculus; ILF, inferior longitudinal fasciculus; Fx, fornix; CC, corpus callosum; AQ, autism spectrum quotient

Figure 1

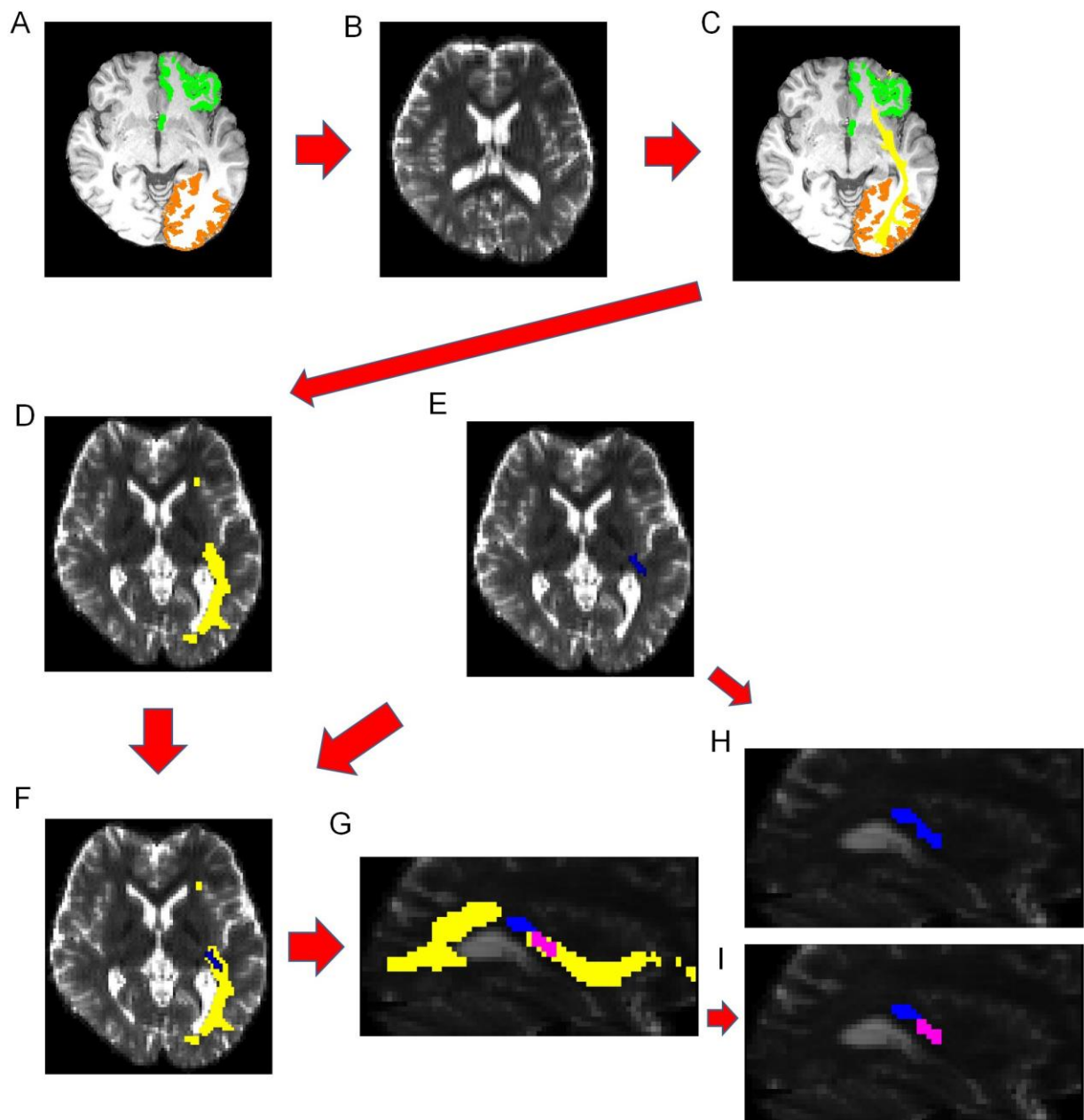


Figure 1

The scheme of tracking fibers and identifying locations of TBSS clusters.

A, A 3D-MPRAGE structural image of an individual brain with seeds obtained from FreeSurfer cortical parcellation.

B, A B0 image. Probtrackx tracked fibers in diffusion space, using seeds defined in A.

C, A structural image with seeds and an identified fiber. Probtrackx produces the results in the same space as seeds.

D, The fiber tract transferred into diffusion space.

E, A significant cluster from TBSS, transformed from MNI152 space into each subject's diffusion space.

F, The fiber tract overlapped with TBSS cluster.

G, The fiber tract and TBSS cluster in the sagittal plane. The overlapped region is colored in magenta.

H, A TBSS cluster in the sagittal plane. We call this the Cluster of Interest.

I, A TBSS cluster with the overlapped part in the sagittal plane. We call magenta part as the Overlap.

TBSS, tract-based special statistics; MNI, Montreal Neurological Institute

Figure 2

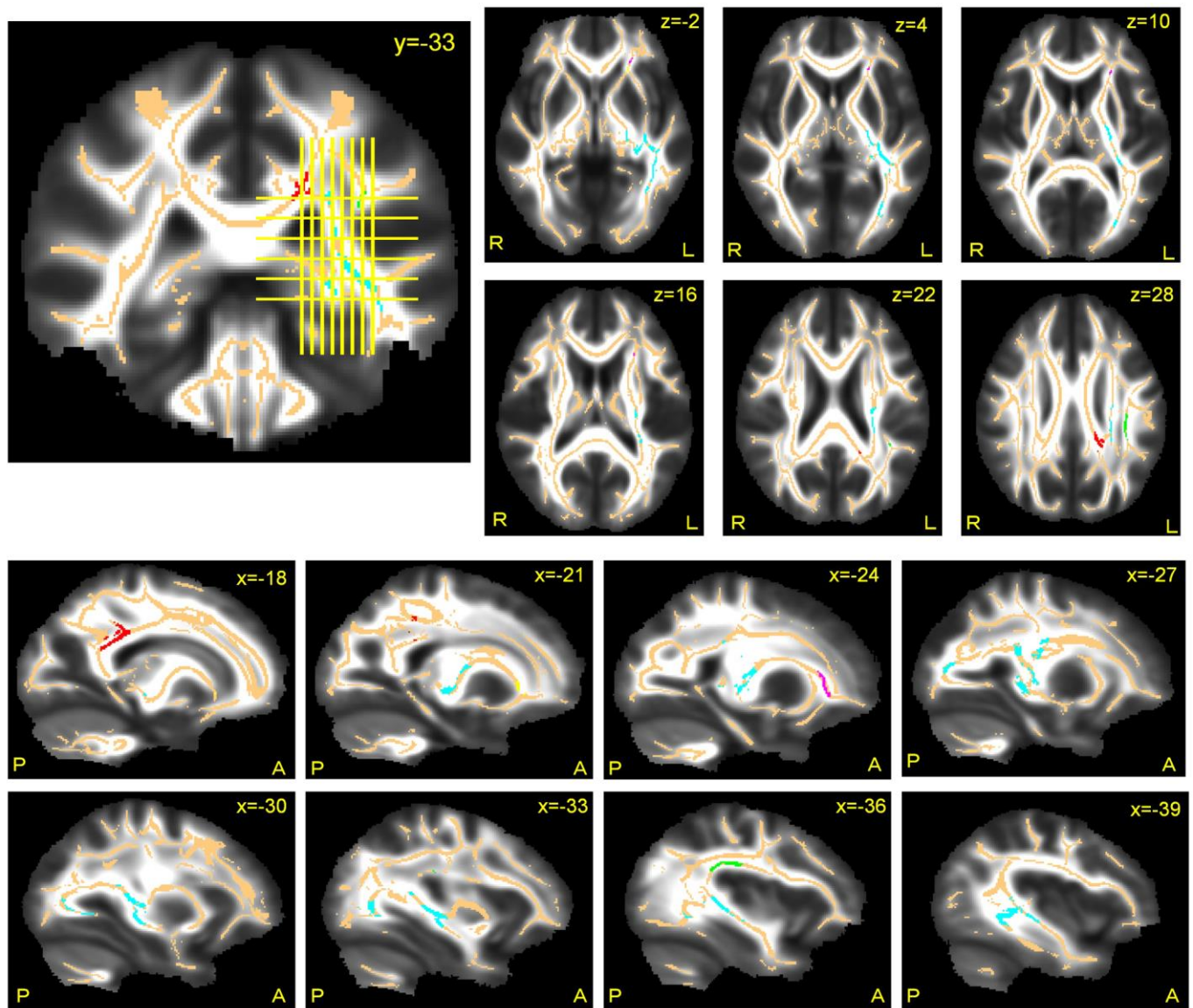


Figure 2

Images of all clusters in standardized brain with FA values negatively correlated with AQ. Colors change by clusters. Copper-colored region is skeleton mask. Yellow lines in the left upper picture show the position of each slice. Light blue region is the largest cluster, and red is the second largest. The 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> clusters are green, purple and yellow, respectively. R: right, L: left, P: posterior, A: anterior, FA: fractional anisotropy, AQ: autism spectrum quotient,

Figure 3

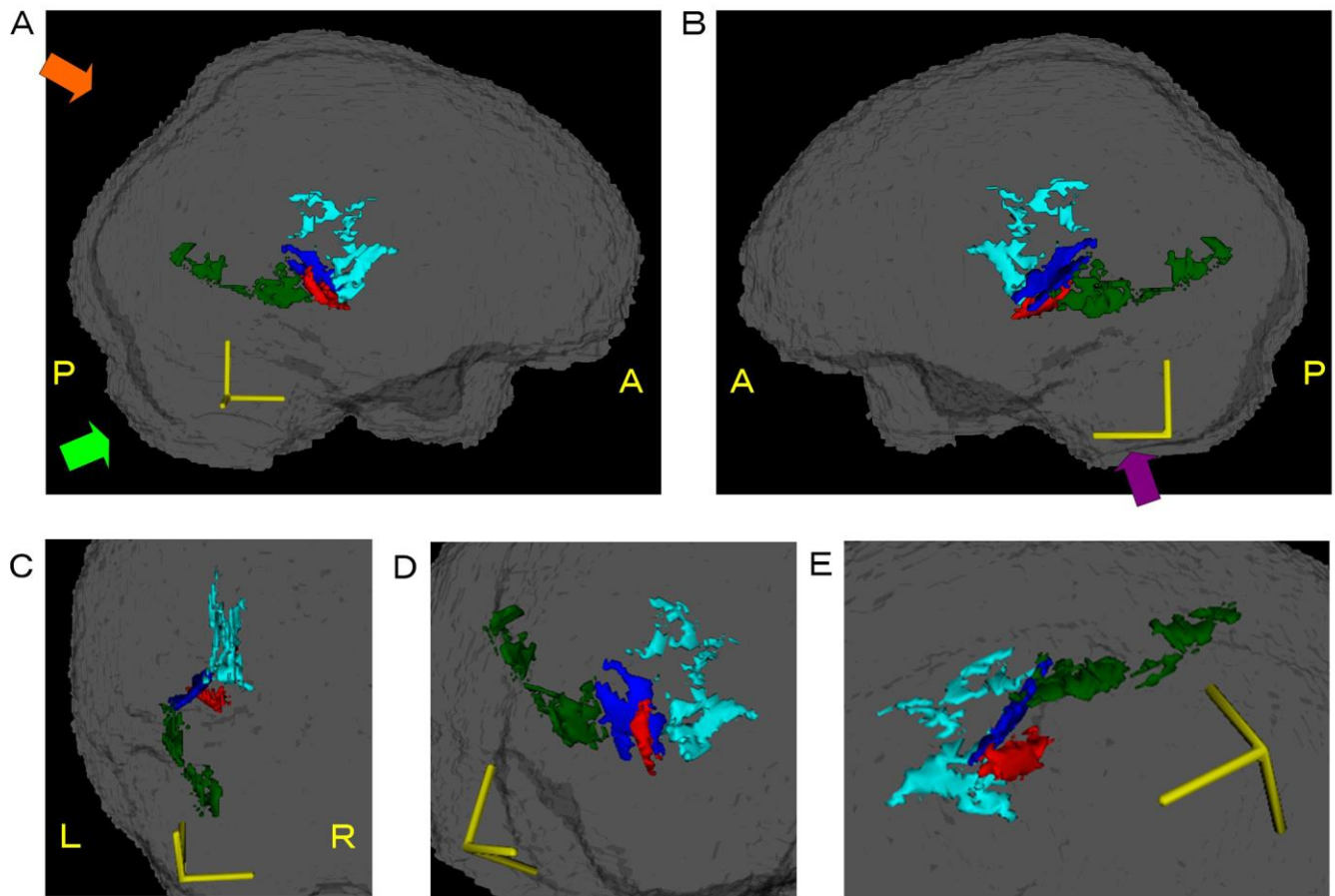
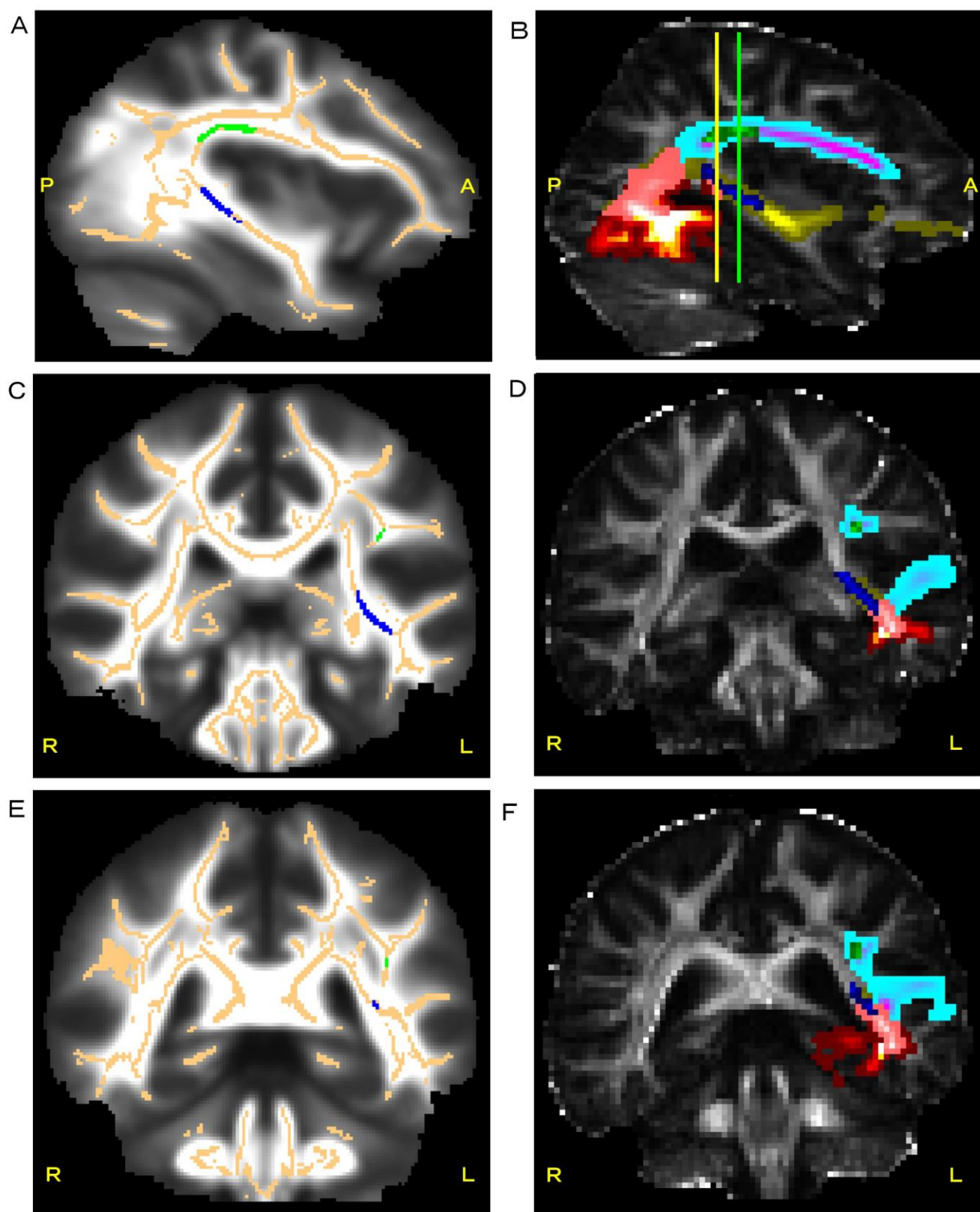


Figure 3

Colored 3D images of the light blue cluster of interest from Figure 1 as seen on standardized brain. Green, red, light blue and blue regions comprise this largest cluster correlated negatively with AQ in TBSS. These 4 subclusters represent occipital part (green), fornix part (red), corona radiata part (light blue) and blue colored region. The blue region was taken away from the whole cluster manually because it was located adjacent to pSTS. Yellow bars are axes on MNI coordinates. A, Image viewed from right side. Orange arrow shows C and light green arrow shows D. B, Image viewed from left side. Bigger clusters than A can be seen because they are located in the left hemisphere. Purple arrow shows E. C, Partial image viewed from posterior upper position. Green part is located far from light blue part. D, Partial image viewed from left backward position. Green part has a gap with blue part. Light blue part also has a gap with blue part. E, Partial image viewed from lower right position. Blue part is contiguous with red part only by 1 voxel. R: right, L: left, P: posterior, A: anterior, 3D: 3-dimensional, AQ: autism spectrum quotient, TBSS: tract-based spatial statistics, pSTS: posterior superior temporal sulcus, MNI: Montreal Neurological Institute

Figure 4



**Figure 4**

Images of clusters and fiber tracts found by TBSS and tractography. A, Sagittal slice showing cluster on pSTS. B, Picture of individual's brain showing similar slice to A and projected on the results of tractography. Cool colors (light blue and purple) show AF. Hot colors (red and white) show ILF. Yellow band shows IFOF. Magenta color shows overlap between ILF and IFOF. Blue is ROI and green is 3rd cluster. Green line shows coronal slices C and D. Yellow line shows E and F. C, Coronal slice on standardized brain. D, Coronal slice on individual's FA image. Blue cluster is not on AF, as AF is more lateral going downward. E, Another coronal slice. F, Another coronal slice on individual's FA image. Blue cluster is on IFOF, not ILF. TBSS, tract-based special statistics; pSTS, posterior superior temporal sulcus; AF, arcuate fasciculus; ILF, inferior longitudinal fasciculus; IFOF, inferior fronto-occipital fasciculus; ROI, region of interest; R, right; L, left; P, posterior; A, anterior, FA: fractional anisotropy,