High angular resolution diffusion imaging in a child with autism spectrum disorder and comparison with his unaffected identical twin

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Summary

In recent years, the use of brain diffusion MRI has led to the hypothesis that children with autism spectrum disorder (ASD) show abnormally connected brains. We used the model of disease-discordant identical twins to test the hypothesis that higher-order diffusion MRI protocols are able to detect abnormal connectivity in a single subject.
We studied the structural connectivity of the brain of a child with ASD, and of that of his unaffected identical twin, using high angular resolution diffusion imaging (HARDI) probabilistic tractography. Cortical regions were automatically parcellated from high-resolution structural images, and HARDI-based connection matrices were produced for statistical comparison. Differences in diffusion indexes between subjects were tested by Wilcoxon signed rank test. Tracts were defined as discordant when they showed a between-subject difference of 10 percent or more.
Around 11 percent of the discordant intra-hemispheric tracts showed lower fractional anisotropy (FA) values in the ASD twin, while only 1 percent showed higher values. This difference was significant.
Our findings in a disease-discordant identical twin pair confirm previous literature consistently reporting lower FA values in children with ASD.

KEY WORDS: abnormal connectivity, autism, connectome, correlation matrix

Introduction

The autism spectrum disorders (ASDs) are a group of complex neurodevelopmental disorders characterized by social and communication impairments and restricted and repetitive interests (American Psychiatric Association, 2013). Although the pathogenesis of autism is still unknown, there is substantial agreement that it has a multifactorial origin involving interaction between multiple susceptibility genes, epigenetic effects and environmental factors (Currenti, 2010).
Recent concurrent evidence suggests that a disruption in brain connectivity is present in ASD individuals, mainly consisting of diffuse cortical under-connectivity (Aoki et al., 2013; Kana et al., 2011; Travers et al., 2012). It is not yet clear whether this disrupted connectivity represents the state-independent endophenotype of the disease (i.e. a genetically determined hallmark of vulnerability) or whether it is, rather, an actual marker of disease manifestation (i.e. a biological correlate of the first signs and symptoms of the disease) (Gottesman and Gould, 2003). Were this latter notion found to be correct, it may prove very useful in leading to more specific diagnostic procedures and effective resource allocation for early intervention.
A model that can be proposed to help disentangle the contribution of genetic make-up from the influence of epigenetic and environmental factors is that of disease-discordant identical twins, i.e. monozygotic (MZ) twin pairs in which only one individual presents with the disease. To date, no studies have used this twin model in ASDs to shed light on the role of disrupted connectivity in the disease pathogenesis. In the present study we address this issue by analyzing inter-individual differences in brain connectivity between a child with ASD and his unaffected identical twin using a higher-order diffusion MRI approach.

Methods

Two MZ male twins (S1 and S2) aged five years and two months were referred to our institute in February

2012 for clinical assessment (Table I). They were born preterm at 27 weeks' gestation. At birth, S1 weighed 1125 g; his length was 35.5 cm and his head circumference was 25 cm. S2 weighed 1000 g; he was 35 cm long and had a head circumference of 25 cm. Brain ultrasounds showed a transient flare, which normalized within the 10th day of life, in both subjects. At term-equivalent age, brain ultrasounds were confirmed as normal. The twins were raised in the same home and history taking revealed no notable differences in environmental exposure. In both children, growth measures had always been within the normal range.

Following admission to our Child Neurology Unit both twins underwent a five-day neurodevelopmental observation, involving clinical evaluation and administration of standardized tests, including the Griffiths Developmental Scale (Griffiths, 1976), the Wechsler Pre-school and Primary Scale of Intelligence – Revised (Wechsler, 1990), the Movement Assessment Battery for Children (Henderson and Sugden, 1992), and the Autism Diagnostic Observation Schedule-Generic (Lord et al., 2000; Tancredi et al., 2005).

Screening for homozygosity was performed by means of i) genome sequence identity of the following loci: CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D81179, D13S317, D16S539, D18S51, D21S11, D19S433, FGA, THO1, TPOX, VWA, Amg, ii) polymerase chain reaction analysis of 15 microsatellites, and iii) fragment analysis performed using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, California).

Brain MRI was obtained using a 1.5T scanner (General Electric Medical Systems, Milwaukee, Wisconsin), an examination that is part of our institute's diagnostic protocol for children with neurodevelopmental delay of unknown origin. Both patients received inhalational anesthesia with an odorless oxygen and nitrous mixture for induction and sevoflurane for maintenance. No side effects were reported. A high-resolution T1 structural image was acquired for each participant together with a high angular resolution diffusion imaging (HARDI) sequence (30 directions, b=1000 s/mm^2) (Tournier et al., 2012). An intensity-based normalization software suite called Advanced Normalization Tools (http://stnava.github.io/ANTs) (Avants et al., 2008; Avants and Gee, 2004) was used to perform motion correction of diffusion-weighted images. For this purpose, a cross correlation similarity matrix for rigid affine transformation was used (Rose et al., 2012). Cortical parcellation was performed on structural images using the Freesurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu), which parcellates the cortex into 33 units per hemisphere on the basis of gyral and sulcal structure, and can be reliably used in children as young as four years of age (Ghosh et al., 2010).

The fiber orientation distribution was estimated using constrained spherical deconvolution with MRtrix software (https://github.com/MRtrix3/mrtrix3/wiki) (Tournier et al., 2012), which allows resolution of crossing fibers and hence more accurate tractographic delineation of white matter pathways (Jones, 2008). Fiber tracking was also performed with MRtrix. Five million probabilistic streamlines were generated seeding randomly over the entire brain volume to create a whole-brain tractogram. Information from the Freesurfer cortical parcellation was combined with tractography information to obtain a connection matrix. The terminal end points of every streamline were hit-tested with every cortical region. Only connections containing at least 250 streamlines were retained for further analysis, and weighted mean fractional anisotropy (FA) and mean diffusivity (MD) were calculated within these connections. Figure 1 outlines the automated image-processing pipeline used in this study.

The study was approved by our institutional ethics committee and written informed consent was obtained from the boys' parents.

Statistical analysis

With the aim of testing whether differences in diffusion indexes (FA) of S1 and S2 were random or whether they showed, rather, a shift toward overexpression or lower expression, FA values in S1 and S2 were compared by Wilcoxon signed-rank test, which is a non-

Table I - Clinical characteristics of the two twins.

	S1 (ASD)	S2 (non-ASD)
Age	5 years 2 months	5 years 2 months
GA	27 weeks	27 weeks
BW	1125 g	1000 g
Length	35.5 cm	35 cm
HC	25 cm	25 cm
DQ	35	87
Language*	Level 1 - Preverbal	Level 4 - Sentences

Abbreviations: GA=gestational age, BW=birth weight; HC=head circumference; DQ=developmental quotient. *Assessed according to Tager-Flusberg et al., 2009

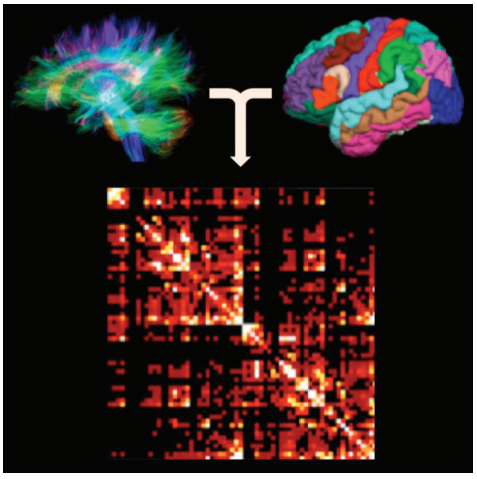

Figure 1 - Automated image-processing pipeline used.
A whole-brain tractography map is generated (left). The cortex is parcellated into 66 regions (right). A matrix representing connectivity parameters (e.g. FA, MD, number of streamlines) between each pair of cortical regions is produced (center).

parametric test that compares two dependent observations and counts the number of negative and positive differences. The number of discordant tracts, defined as those showing a between-subject difference of 10 percent or more, was counted.

Results

The main clinical characteristics of the subjects are reported in table 1. Structural images of S1 and S2 are shown in figure 2.

Following clinical observation, S1 received a diagnosis of ASD. His psychomotor development was delayed and he presented moderate intellectual disability (developmental quotient of 35). His speech was limited to vocalizations, unintelligible babbling, echolalia and only rare words. His gross motor functioning was age appropriate while his fine motor coordination was slightly delayed.

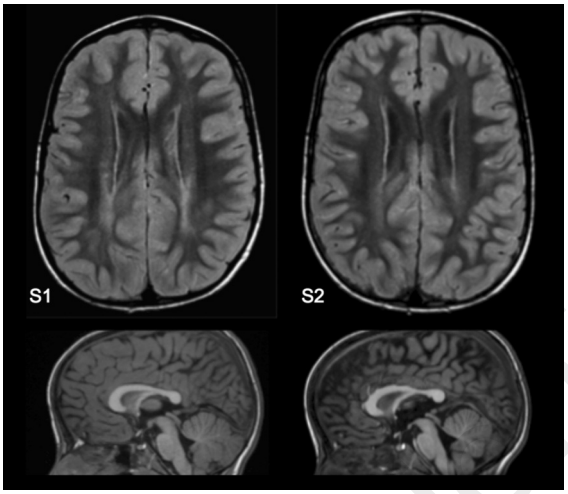

Figure 2 - Structural axial FLAIR (top) and sagittal T1 (bottom) of patients S1 and S2.

Following clinical observation, S2 received a diagnosis of mild language disorder (developmental quotient of 87) at the time of the scan. His psychomotor development was borderline, while his gross motor functioning was age appropriate. S2's behavior was characterized by sub-threshold separation anxiety and social inhibition. At a follow-up assessment at the age of six years, S2 showed a positive evolution with a complete normalization of the clinical picture in terms of language development as well as motor function.

Parcellation of the two brains and HARDI processing were successful. Visual examination of the images did not show differences in movement artifacts and distortions.

Comparison of the twins' data referring to various regions revealed differences as shown in figure 3. Of the 1122 intra-hemispheric tracts analyzed, 136 showed discordant FA values. In 125, FA was lower in S1 than in S2 (ASD under-connected), while in 11 FA was lower in S2 compared to S1 (ASD over-connected), these results suggesting a higher number of under-connected regions in the ASD subject (Wilcoxon signed rank test, $p<0.0001$). Table II details the distribution of the intra-hemispheric tracts showing discordance. Around eleven percent of the discordant intra-hemispheric tracts were ASD under-connected (15.2% of the intra-lobar and 9.3% of the inter-lobar), while only 1% of the discordant intra-hemispheric tracts were ASD over-connected (2% of the intra-lobar and 0.5% of the inter-lobar). Only six discordant tracts were inter-hemispheric.

Reverse results were observed when plotting MD values (not shown).

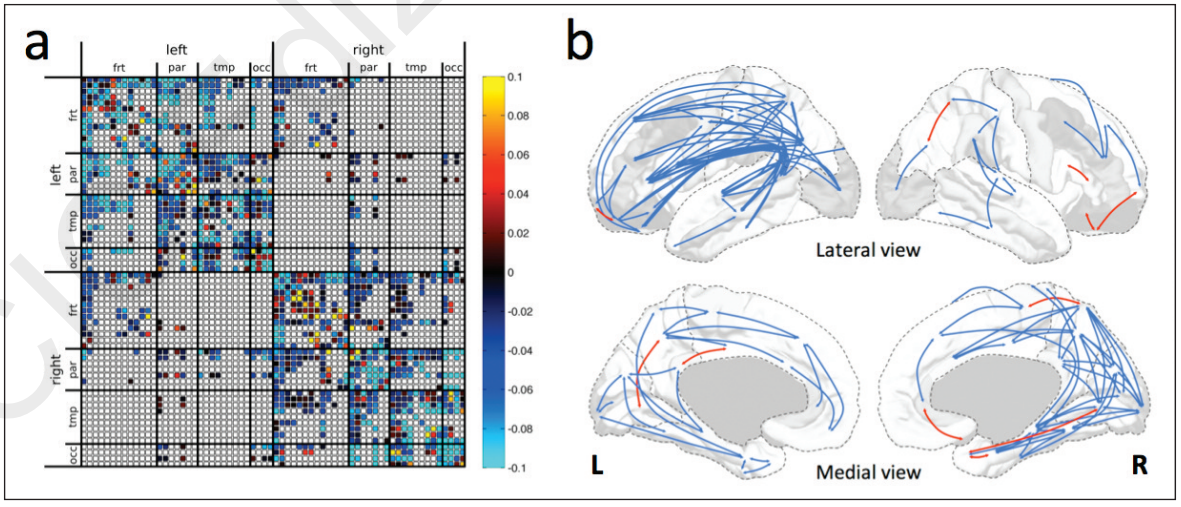

Figure 3 – Connection matrix and topographic distribution of FA discordant pathways

a) Connection matrix based on differences in FA values between the two subjects (S1–S2) / 0.5*(S1+S2). Light blue indicates connected regions where FA values were at least 10% lower in the ASD subject than in the control. Light yellow indicates connected regions where the FA values of the ASD subject were at least 10% higher than those of the control. b) Schematic representation of FA discordant pathways between the two subjects, represented as arrows connecting the two regions (the direction of the path is arbitrary). Blue arrows indicate connected regions where the FA values of the ASD subject were at least 10% lower than those recorded in the control while red arrows indicate connected regions where the FA values of the ASD subject were at least 10% higher than those of the control.

Table II - Distribution of discordant tracts.

	Total number of intra-hemispheric tracts	ASD under-connected tracts % of the total (% of the under-connected)	ASD over-connected tracts % of the total (% of the over-connected)
INTRA-LOBAR			
F-F	182	10.4 (15.2)	2.7 (45.5)
P-P	56	23.2 (10.4)	1.8 (9.1)
T-T	90	17.8 (12.8)	1.1 (9.1)
O-O	20	25.0 (4.0)	0
Total intra-lobar	348	15.2	2.0
INTERLOBAR			
F-P	182	12.1 (17.6)	1.1 (18.2)
F-T	234	6.0 (11.2)	0
F-O	104	2.9 (2.4)	0
P-T	126	6.3 (6.4)	0
P-O	56	23.2 (10.4)	1.8 (9.1)
T-O	72	16.7 (9.6)	1.4 (9.1)
Total inter-lobar	774	9.3	0.5
Total	1122	11.1	1.0

Abbreviations: F=frontal; P=parietal; T=temporal; O=occipital; ASD=autism spectrum disorder

Discussion

The distribution of corticocortical connections showing different FA values between our twins (discordant tracts), and specifically our finding of a significant majority of discordant tracts (94.2%) having lower FA values in the ASD twin, is broadly consistent with previous literature reporting lower FA values in children with ASD. Most studies show that structural connectivity between brain regions is weaker, in terms of lower FA values, in individuals with ASD compared to controls. Reduced FA of white matter has been reported as a global feature of the ASD brain, particularly in the frontal and temporal cortex, in the main fasciculi connecting different lobes, in subcortical regions and in the corpus callosum [see (Vissers et al., 2012) for a review]. Our study is mostly consistent with these reports, in that ASD tracts with lower FA values were found both within the intra-lobar connections (with percentages ranging from 10 to 25% in the four lobes) and within the inter-lobar connections, and in particular in the parieto-occipital, temporo-occipital and frontoparietal tracts.

The fact that the great majority of discordant tracts observed in our twins were located in the left hemisphere, with discordance involving the frontoparietal and frontotemporal connections in particular (see Fig. 3b), suggests a possible reduction of FA left lateralization in the ASD twin. Indeed, one of the emerging biological hallmarks of ASD is the loss or inversion of the typical patterns of brain lateralization, which involve, in particular, but are not limited to, the language-related frontotemporal networks (Toga and Thompson, 2003). Recent diffusion imaging and tractography studies in adolescents and young adults with high-functioning ASD have provided reports of loss or inversion of the left-right asymmetry in the cingulate, arcuate fasciculus and uncinate fasciculus (Knaus et al., 2010; Lo et al., 2011; Fletcher et al., 2010), in the superior temporal gyrus (Lange et al., 2010), and in the pathways involving the fusiform (Conturo et al., 2008). Converging evidence has also been provided by functional MRI studies showing reduced asymmetries in brain representation of language-related networks (Eyler et al., 2012).

A small number of discordant tracts in our study showed higher FA values in the ASD twin. Increased connectivity values have previously been reported in individuals with ASD, particularly within the frontal lobe, using functional and structural methods. For example, increased functional connectivity was found in the frontostriatal circuitry in adolescents and young adults with ASD (Delmonte et al., 2013), a finding consistent with earlier studies (Di Martino et al., 2011). Increased connectivity in the frontal regions has been interpreted as a compensatory mechanism for global under-connectivity (Kana et al., 2011). Although it needs to be underlined that our statistical analysis only supports the presence of a significantly higher number of tracts with lower FA values in the ASD twin, it is of interest that in our two patients almost half (46%) of the tracts with higher FA values were found within the frontal lobe, versus only 15% of the ones showing lower FA values.

Low FA values have been widely interpreted as an index of weak structural connectivity, thus reflecting functional evidence of a pattern of under-connectivity in ASD (Vissers et al., 2012). However, it needs to be underlined that diffusion data, above all FA data, may not be interpreted as a direct measure of structural connectivity (Castellanos et al., 2014; Jones et al., 2013).

A potential bias in our analysis is that S2, the non-ASD twin, did not show a psychomotor profile fully typical for a healthy subject, as he received a diagnosis of mild language disorder, and showed some sub-threshold separation anxiety and social inhibition. As some reports support partially overlapping neurophysiological underpinnings of language impairment and ASD (Verly et al., 2014), it might be suggested that the dis-

cordance in our twins is an underestimation of the real differences between ASD and typical brains. It should also be noted that the analysis could be affected by the presence of white matter abnormal signal intensity in both subjects, likely related to preterm birth. Indeed, cerebral white matter abnormalities on structural MRI have been extensively described in infants born prematurely, in particular punctate high-signal intensities on T1-weighted images and diffuse high-signal intensities on T2-weighted images (Rutherford et al., 2010).

A very low birth weight (<1500 g) as well as prematurity have repeatedly been identified as risk factors for an ASD (Elgen et al., 2002; Indredavik et al., 2010; Limperopoulos et al., 2008). In fact, the prevalence of ASD among children born prematurely is between 3.65% and 8% (Hack et al., 2009; Johnson et al., 2010; Pinto-Martin et al., 2011), compared to 1% in the general population of school-aged children (Baron-Cohen et al., 2009). Future investigations should attempt to determine the role of these neonatal and perinatal risk factors in the development of ASD.

This study is the first attempt to use the model of discordant twins to explore the role of abnormal connectivity in the pathogenesis of ASD. In a recent longitudinal study on 92 high-risk infant siblings, 28 of which were eventually diagnosed with ASD, the FA trajectories, from six to 24 months, for several fiber tracts differed significantly between the infants who developed ASD and those who did not, suggesting that aberrant white matter development may precede the manifestation of autistic symptoms, thus representing a reliable biomarker of the disease (Wolff et al., 2012). Since the affected subjects shared only some of the genetic heritage of their unaffected siblings, it could not be determined whether this biomarker is more the direct result of genetic determination predisposing to ASD, or rather an early sign of a multifactorial process (genetic, epigenetic and environmental) leading to disease expression. Our findings are more in favor of the latter. Since our twins share the same genetic heritage, the differences in brain connectivity, which are consistent with previous studies, suggest that abnormal connectivity as a biomarker for ASD lies closer to the final steps in the pathogenetic process than to the early ones. Unquestionably, our study represents only a first, preliminary attempt to address these important issues. The model of discordant MZ twins is promising and we expect our preliminary data to foster further research on this topic.

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