Comparing Fractional Anisotropy in Patients With Childhood-Onset Schizophrenia, Their Healthy Siblings, and Normal Volunteers Through DTI

Marcel E. Moran1,3, Zoe I. Luscher1,3, Harrison McAdams1, John T. Hsu2, Deanna Greenstein1, Liv Clasen1, Katharine Ludovici1, Jonae Lloyd1, Judith Rapoport1, Susumu Mori2, and Nitin Gogtay*,1

1National Institute of Mental Health, National Institutes of Health, Bethesda, MD; 2The Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University School of Medicine, Baltimore, MD.

3These authors contributed equally to the article.

*To whom correspondence should be addressed. National Institute of Mental Health, National Institutes of Health, Room 3B19 10 Center Drive, Bethesda, MD 20892, US; tel: 301-435-4494, fax: 301-402-7160, e-mail: gogtayn@mail.nih.gov

Background: Diffusion tensor imaging is a neuroimaging method that quantifies white matter (WM) integrity and brain connectivity based on the diffusion of water in the brain. White matter has been hypothesized to be of great importance in the development of schizophrenia as part of the dysconnectivity model. Childhood-onset schizophrenia (COS), is a rare, severe form of the illness that resembles poor outcome adult-onset schizophrenia. We hypothesized that COS would be associated with WM abnormalities relative to a sample of controls. Methods: To evaluate WM integrity in this population 39 patients diagnosed with COS, 39 of their healthy (nonpsychotic) siblings, and 50 unrelated healthy volunteers were scanned using a diffusion tensor imaging (DTI) sequence during a 1.5 T MRI acquisition. Each DTI scan was processed via atlas-based analysis using a WM parcellation map, and diffeomorphic mapping that shapes a template atlas to each individual subject space. Fractional anisotropy (FA), a measure of WM integrity was averaged over each of the 46 regions of the atlas. Eleven WM regions were examined based on previous reports of WM growth abnormalities in COS. Results: Of those regions, patients with COS, and their healthy siblings had significantly lower mean FA in the left and right cuneus as compared to the healthy volunteers (P < .005). Together, these findings represent the largest DTI study in COS to date, and provide evidence that WM integrity is significantly impaired in COS. Shared deficits in their healthy siblings might result from increased genetic risk.

Key words: DTI/COS/siblings/cuneus/FA

Introduction

Mounting evidence suggests that the core pathophysiology of schizophrenia results not from a focal brain lesion, but rather from abnormal connectivity or communication between discrete brain regions.1,4 This so-called dysconnectivity model of schizophrenia was first formalized by Friston and Frith in 1995,5 when they observed aberrant prefrontaltemporal connectivity in schizophrenia patients using positron emission tomography (PET). A modern take on the dysconnectivity model is more generalized, emphasizing “the role of the brain’s integrative processes—the substrate of which is connectivity—in the pathophysiology of schizophrenia.”6 Studying childhood-onset schizophrenia (COS), defined by onset of psychosis before the age of 13, represents a unique opportunity to contribute to this research. With psychosis onset occurring during childhood, COS can give special insight to the neurodevelopmental trajectory of schizophrenia.3 Due to greater genetic loading8 and reduced risk of institutionalization or substance abuse confounds,9 COS may also help elucidate the etiology of schizophrenia as a whole. Since the beginning of the NIMH COS project in 1990, COS has been documented as symptomatically continuous with the more common adult-onset schizophrenia (AOS),10 as well as sharing similar brain abnormalities such as increased ventricular volume11 and decreased gray matter volume.12 Less has been studied regarding WM in COS, although longitudinal growth rates have been documented to be significantly slower than those in normal volunteers.4

One way to address the dysconnectivity model in schizophrenia is through analysis of white matter (WM) organization, probing the structural basis of the brain’s connections. Diffusion tensor imaging (DTI), a magnetic resonance image technology that uses the diffusion of water in the brain to detect neural architecture, has the ability to characterize WM integrity at a level never before
possible. This tool has opened up widespread research into WM and its involvement in neurological and psychiatric disorders. The most prominent WM abnormalities emerging from the growing body of DTI studies of schizophrenia implicate the frontal lobe, and specifically frontal, frontotemporal, and frontalimbic connections. Similarly, the most consistently reported tracts include the superior longitudinal fasciculus, corpus callosum, uncinate fasciculus, and cingulum. Although a consensus is beginning to build, results from different studies are not entirely consistent and the number of brain regions showing WM differences continues to increase.

Examining the DTI literature for COS and early-onset schizophrenia (EOS, defined as onset before the age of 18) reveals an equally nonspecific picture of dysconnectivity. Many studies highlight similar connectivity deficits involving the frontal lobe, including results showing WM abnormalities in the bilateral prefrontal lobes, corpus callosum, right superior longitudinal fasciculus, left inferior longitudinal fasciculus, and left inferior frontooccipital fasciculus. Additional studies reveal WM deficits in regions commonly associated with schizophrenia and executive functioning, namely the left posterior hippocampus and the left anterior cingulate. Yet these results represent only a partial list of DTI findings; as with AOS, the growing body of literature suggests a more heterogeneous story. This variability is partially explained by different methodological considerations, such as differing scan parameters or choosing to perform a whole brain as opposed to hypothesis driven analysis. Another limiting factor for COS and EOS studies in particular is the rarity of subjects, and many studies to date rely on small samples that can limit statistical power.

We have also chosen to include healthy siblings of COS patients in the present analysis, an approach, in new to DTI studies of COS and EOS. As COS subjects and their siblings are likely to share genetic and early life environments, comparison of COS subjects to their siblings in addition to healthy controls could better resolve abnormalities specific to the development of schizophrenia. In addition, some findings from AOS DTI research demonstrate an intermediate WM integrity phenotype in unaffected siblings as opposed to AOS subjects and controls. Further examination of these differences in COS is all the more critical, as the largest review of siblings of patients with schizophrenia to date has indicated that the most detectable differences in siblings are most likely those that happen earlier in life. Additional investigation of structural abnormalities that differentiate affected families from those of subjects without histories of psychiatric disorder could spotlight specific brain regions for further research regarding endophenotypes for schizophrenia.

To explore the nature of WM integrity in COS and their siblings, 50 patients with COS, 41 of their healthy (nonpsychotic) siblings, and 50 healthy, nonrelared volunteers underwent a 1.5 T MRI scan with a DTI sequence. These images were processed via atlas-based analysis that creates a participant-specific brain atlas and provides mean FA measures of 46 different WM regions. Based on our group’s previous tensor-based morphometry findings detailing specific WM abnormalities in COS, we limited our hypothesis to the 11 most affected atlas regions.

Given the consistent brain abnormalities present in the COS population and the theorized and documented associations between schizophrenia and disrupted WM, we hypothesized that patients with COS would have decreased FA as compared to their healthy siblings and nonrelated volunteers. Based on work indicating structural brain differences as an endophenotype in schizophrenia, we also hypothesized that siblings of patients with COS would exhibit significantly decreased FA compared to unrelated healthy volunteers. To our knowledge, this is the largest study of DTI in COS.

Methods

Participants

All participants involved in this study were recruited as part of the ongoing NIMH COS and Normal Brain Development studies. Since 1990 over 3000 cases of potential COS have been submitted to the study, of which over 300 were invited to the NIH Clinical Center for a formal screening and evaluation. These procedures were followed by a 2–3-week drug-free inpatient observation period. During this period, we obtained measures of symptom severity, including the Scale for the Assessment of Positive Symptoms (SAPS, intraclass correlation coefficient for total score = 0.834), the Scale for the Assessment of Negative Symptoms (SANS, Chronbach a for total score = 0.885), and Children’s Global Assessment Scale (CGAS) scores. Inclusion criteria were presence of psychosis before the child’s 13th birthday, premorbid IQ of 70 or above, and absence of significant neurological illness. The screening process has been described in further detail elsewhere. Following a subject’s diagnosis of COS, his or her full siblings were enrolled in prospective neuroimaging. We conducted diagnostic interviews with siblings over 18 years using the Structured Clinical Interview for the DSM-IV (SCID) and in siblings younger than 18 and/or their parents using Kiddie-Schedule for Affective Disorder and Schizophrenia (K-SADS). Siblings over 14 years underwent DSM Axis II personality disorder interviews with trained interviewers using the Structured Interview for DSM-IV Personality (SID-P). Inter-rater reliability was excellent (average κ = 0.90). All siblings with a schizophrenia spectrum disorder were excluded from this analysis.

Healthy volunteers were recruited from the community, and were also screened for neurological and psychiatric illness. Details about subject recruitment have been previously described. A group of healthy controls was selected from a larger sample to match the COS and sibling groups on age, sex, race, and handedness. Healthy
volunteer selection was performed while blind to all MRI-based measurements. Informed consent from all participants older than 18 and assent from all minor participants were obtained either from participants or legal guardians. The study was approved by the NIMH IRB.

We compared groups on age, sex, race, handedness, socioeconomic status, as measured using the Hollingshead A. B. (unpublished manuscript, 1975) and expressive vocabulary as measured by age-appropriate versions of the Vocabulary subtest from the Wechsler intelligence scales. Demographic data is provided in table 1.

**Image Acquisition**

Participants were scanned on a 1.5-T MRI scanner (Signa; General Electric, Milwaukee, WI, USA) at the National Institutes of Health Clinical Center in Bethesda, Maryland. The diffusion-weighted images were gathered from two sequences (repetition time/echo time = 17 s/65 s, six gradient directions).

**Data Processing and Analysis**

The DTI images were processed via atlas-based analysis, in which a region-by-region map template is shaped onto each individual subject (figure 1). Initially, the raw diffusion weighted images were coregistered to one of the least diffusion-weighted images, and a 12-mode affine transformation of Automated Image Registration was used to correct motion and eddy current distortion. Some subject scans were removed from the sample due to low contrast or movement, reducing the sample size to 39 patients with COS, 39 siblings, and 50 healthy volunteers. All images underwent a manual and automated skull stripping to remove pieces of the skull and sinuses still visible in the diffusion weighted images. Next, large deformation diffeomorphic metric mapping (LDDMM) and inverse linear matrix were use to warp the parcellation map from the template into each participant’s subject space, using the software program Diffeomap. (www.mristudio.org). These steps in the LDDMM pipeline have been described in greater detail elsewhere. For the atlas-based analysis, a single subject template was used, which is segmented into 176 3D parcels. Specifically, a type II WM parcellation map atlas was used that parcelates the cortex and associated superficially located WM together with a 0.2 threshold. From the list of all parcellations, we identified the 11 regions of interest showing aberrant WM growth trajectories in COS, including the left and right cuneus, the left and right superior frontal gyrus, the left and right precuneus, the left and right cingulate gyrus, the left and right middle frontal gyrus, and the right cerebellum. We processed WM scans while blinded to group membership, and we included probands, siblings, and controls in each processing batch to ensure any systematic changes in skull stripping accuracy did not impact one or two of the three groups.

Because the sibling and COS groups were not independent (ie we had multiples siblings per family and probands and siblings from the same family), we tested group differences for each region and average FA using a mixed effect regression model. Our fixed effects included group (levels = COS, sibling, control), age at scan, and sex. A random intercept per family allowed us to estimated variability within family as a variance parameter. Our statistical model is as follows:

\[ y_{ij} = b_0 + b_1 \text{(group = sib)} + b_0 \text{(group = COS)} + b_1 \text{(age, i)} + d_i + e_{ij} \]

where \( y_{ij} \) is the FA value from \( i \)th person from the \( j \)th family, \( d_i \) is the random intercept for the \( j \)th family, and \( e_{ij} \) is the normally distributed random error (\( e_{ij} \) and \( d_i \) are iid \( \sim N(0, \sigma^2) \)). The intercept (\( b_0 \)) is the estimated mean of the control group.

To correct for multiple comparisons, we used the false discovery rate and set \( q = 0.05 \). We screened the data for outliers and explored the normality assumption using qqplots and histograms of residuals. Outliers were defined using the \( P \) value associated with the standardized residual for each model/region of interest: if \( P < .05 \) (corrected for multiple comparisons), the case was considered an outlier.

**Table 1. Participant Demographics**

<table>
<thead>
<tr>
<th></th>
<th>HV (N = 50)</th>
<th>COS (N = 39)</th>
<th>COS sibs (N = 39)</th>
<th>COS vs HV</th>
<th>COS sibs vs HV</th>
<th>ANOVA</th>
<th>F (P)</th>
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<tr>
<td>Sex (F:M)</td>
<td>19:31</td>
<td>15:24</td>
<td>17:22</td>
<td>.03 (1)</td>
<td>.01 (1)</td>
<td>.75</td>
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<tr>
<td>Race (AA:O:W)</td>
<td>11:8:31</td>
<td>9:5:25</td>
<td>9:3:27</td>
<td>.18 (2)</td>
<td>.14 (2)</td>
<td>.5</td>
<td>—</td>
</tr>
<tr>
<td>Handedness M:L:R</td>
<td>0:5:45</td>
<td>2:2:35</td>
<td>2:2:33</td>
<td>.32 (2)</td>
<td>.32 (2)</td>
<td>.2</td>
<td>—</td>
</tr>
<tr>
<td>Mean IQ (SD)</td>
<td>112.1 (14.8)</td>
<td>81.3 (16.4)</td>
<td>104.1 (10.1)</td>
<td>.87 (80)</td>
<td>.96 (2)</td>
<td>.05</td>
<td>F = 43.6 &lt;.0001</td>
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<tr>
<td>Mean SES (SD)</td>
<td>43.9 (19.3)</td>
<td>56.9 (29.4)</td>
<td>45.1 (21.4)</td>
<td>1149.5</td>
<td>934.5</td>
<td>.99</td>
<td>F = 3.80 .025</td>
</tr>
<tr>
<td>Mean age (SD)</td>
<td>19.3 (6.3)</td>
<td>19.7 (5.7)</td>
<td>18.1 (7.4)</td>
<td>.28 (87)</td>
<td>.35 (87)</td>
<td>.73</td>
<td>F = 0.178 .84</td>
</tr>
</tbody>
</table>

*Note: W, White; AA, African America; O, other; M, mixed; L, left handed; R, right handed; SES, socioeconomic status; HV, healthy volunteers.*
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Results

COS vs Healthy Controls

Among the 11 different brain regions (5 regions bilaterally and the right cerebellum; see figure 2 for sample sagittal plots) analyzed individually using mixed model regression, mean FA was significantly lower in patients with COS compared to healthy volunteers in the left ($P = .005$) and right cuneus ($P = .0017$). Both left and right cuneus differences survived FDR correction, and the left cuneus difference survived removal of outliers ($FA < 0.25$). There were no other significant regional FA differences between patients with COS and healthy volunteers that survived FDR correction, and there was not a significant difference in mean FA (averaged across 46 regions in the cortex and cerebellum) between COS and healthy volunteers ($P = .09$). See table 2 for group means and mixed model regression results. (supplementary table 1 includes adjusted (based on sex and age) group FA means from mixed model regressions).

Siblings vs Healthy Controls

Siblings of patients with COS also had significantly lower mean FA in the left ($P = .001$) and right cuneus ($P = .0007$) compared to healthy volunteers. These differences survived FDR correction, and the left cuneus difference survived removal of outliers ($FA < 0.25$). FA did not differ between siblings of patients with COS and controls for the remainder of the regions (see table 2). The difference in mean FA (averaged across 46 regions in the cortex and cerebellum) between siblings of patients with COS and healthy volunteers ($P = .03$) did not survive FDR correction.

Correlations with Clinical Measures

In post hoc analyses, we correlated left and right cuneus FA with medication free inpatient measures of positive symptoms (SAPS), negative symptoms (SANS), overall functioning (GAS) and auditory and visual hallucinations (based on SAPS SS36 and SS41, respectively). None of these correlations were statistically significant.

Because WM integrity correlates with measures of IQ in several different studies, we added IQ to the fixed effects of each model in post-hoc analyses. The IQ coefficient was not significant in any of the models. However, the left and right cuneus differences between controls and
COS ($P = .17$ (l), $P = .07$ (r)) and the right cuneus difference between sibs and controls ($P = .10$) were no longer significant when IQ was in the model. While the sib/control left cuneus difference remained statistically significant ($P = .03$) with IQ in the model, this difference would not survive correction for multiple comparisons.

We conducted additional analyses to determine the effects of medication. Specifically, we calculated chlorpromazine equivalents and used this as a continuous variable to predict FA for left and right cuneus while controlling for age and sex. For both left and right cuneus models, the chlorpromazine equivalent coefficient was not significant. We also explored the relationship between antidepressant medication status while controlling for age and sex [11 COS members were on an antidepressant at the time of scan vs 24 who were not (we were missing data for the remainder)]; there were no significant group differences for either left or right cuneus.

Outliers

In exploring normality assumptions, one residual from the right cingulate gyrus model met our outlier criterion. When we deleted the corresponding subject and reran the analysis, the meaning of the results was not altered so we reported results from the full data set. However, we wanted to ensure that the cuneus group differences were not due to the visibly low raw Mean FA values (two for sibs and one for COS) (see figure 3) so we ran the analysis for left cuneus without these points.

Most of the measures did not have serious departures from normality, based on Q-Q plots and histograms of the residuals, except for the left cingulate gyrus. At the same time, linear models are fairly robust to violations of normality (especially given adequate df) and given our sample size, we were not overly concerned about violations. We did attempt transformations (eg log and square root transformations) for several variables (eg left cuneus, right cingulate gyrus), but they did not sufficiently improve the distribution of the residuals.

Discussion

The present DTI analysis revealed decreased FA in all 11 regions of interest examined in COS patients and their siblings relative to normal controls, suggesting reduced WM integrity in these areas. This decrease survived correction for multiple comparisons in the left and right cuneus, for both patients with COS and their healthy siblings.
The different analytical methods employed in various studies complicate the ability to contextualize these results within the broader schizophrenia literature. The current study does not replicate the most prominent findings regarding frontal lobe WM deficits in schizophrenia. In selecting our a priori regions of interest from a previous study on WM growth trajectories in COS, we effectively limited the range of possible results; a closer examination of the frontal lobe in this sample might lead to findings more similar to those found in other studies. Another possibility is that differences in results presented here reflect brain changes unique to COS, rather than AOS. These findings are particularly important for further neurodevelopmental studies of dysconnectivity models in the pathophysiology of schizophrenia, and for endophenotypic studies of neurocircuitry abnormalities.

Part of the intrigue in studying COS is the potential insight into the neurodevelopmental model of schizophrenia, which broadly states that "the illness is the end stage of abnormal neurodevelopmental processes that began years before the onset of the illness." Typical development entails WM growth at least through adolescence, and COS patients exhibit a relatively reduced WM growth rate during this time. Perhaps the deficit in WM integrity measured here in the cuneus results from this aberrant developmental pattern in COS, offering a possible neurodevelopmental mechanism for the observed dysconnectivity.

The cuneus is of particular interest in schizophrenia because of its location in the primary visual cortex. Both the dorsal and ventral visual networks begin in the primary visual cortex, making the region a site of substantial significance to visual perception and processing. Of particular relevance to COS is the association that has been found between deficits in the cuneus and visual hallucinations. A recent analysis of COS symptomatology indicated that visual hallucination rates are significantly higher in COS as opposed to the more common AOS. Though it is currently difficult to fully explicate the method by which reduced FA in the cuneus relates to visual hallucinations, one possible explanation is that disorganized WM connectivity in the visual cortex leads to disorganized processing of real or imagined visual stimuli, so that imagined visual stimuli may be more likely to be perceived by the individual as externally sourced.

Significant FA reductions present in both patients with COS and their unaffected siblings indicate that the integrity of WM in the cuneus might not be a true disease marker of COS, but rather an indicator of genetic risk for the disorder. Although this line of research is exciting, further work is needed in order to draw any substantial conclusions. Some studies show shared FA abnormalities among schizophrenic patients and their healthy siblings in certain brain regions but not others, and others reveal FA increases in healthy siblings relative to normal volunteers. Understanding how genetic risk affects WM and schizophrenia remain crucial, but further analysis is required to delineate heritability, implicated brain regions, direction of FA abnormalities, and changes over time.

The future directions for this line of research are exciting. The WM differences described herein could be further described through the use of higher resolution DTI. Radial diffusivity, axial diffusivity, or tractography analyses in addition to FA comparisons could display a more complete picture of WM integrity in COS. DTI is especially attractive because of how well it can complement other neuroimaging modalities, providing the structural counterpoint to functional connectivity studies using magnetoencephalography or functional MRI. The COS project within the NIMH continues to collect longitudinal DTI data, potentially enabling more sensitive examination of WM changes over time.

We have identified two primary limitations in this study, and the first limitation involves the difference in subject group IQ. In post hoc analyses, addition of IQ to the models eliminated statistical significance of our findings regarding the left and right cuneus differences between controls and COS, as well as the right cuneus difference between siblings and controls. Because IQ is correlated with group (e.g., the COS group has notably lower IQ scores), it is possible that adding IQ removed variance related to group membership. An additional limitation inherent to this work is exposure to antipsychotic medication in patients with COS. Because of the severity of the illness presentation, the possibility for a medication naive population of patients with COS is not possible. Post hoc analyses of medication effects, however, revealed no relationship between medication and FA.

As mentioned, this is a cross sectional study and longitudinal data may indicate developmental differences in WM development that are indicated in previous studies. Overall, this data set demonstrates that patients with COS have significant abnormalities in the organization of their WM tracts, a functional unit of prime importance in the nervous system. That the siblings of patients with COS also show significant differences from healthy volunteers adds weight to the notion that WM structural differences may be involved in the genetic precursors of schizophrenia. Future studies are needed to replicate and expound on this result, as well as connect it to the other imaging modalities currently available in psychiatric and neuroscience research.

Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

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