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FRACTIONAL ANISOTROPY DISTRIBUTIONS IN 2-6 YEAR-OLD CHILDREN WITH AUTISM

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Abstract

Background: Increasing evidence suggests that autism is a disorder of distributed neural networks that may exhibit abnormal developmental trajectories. Characterization of white matter early in the developmental course of the disorder is critical to understanding these aberrant trajectories. **Methods**: A cross-sectional study of 2-6 year old children with autism was conducted using diffusion tensor imaging combined with a novel statistical approach employing fractional anisotropy distributions. 58 children aged 18-79 months were imaged: 33 were diagnosed with autism, 8 with general developmental delay (DD), and 17 were typically developing (TD). Fractional anisotropy values within global white matter, cortical lobes, and the cerebellum were measured and transformed to random *F* distributions for each subject. Each distribution of values for a region was summarized by estimating δ, the estimated mean and standard deviation of the approximating *F* for each distribution. **Results**: The estimated δ parameter, $\hat{\delta}$, was significantly decreased in individuals with autism compared to the combined control group. This was true in all cortical lobes, as well as in the cerebellum, but differences were strongest in the temporal lobe. Predicted developmental trajectories of $\hat{\delta}$ across the age range in the sample showed patterns that partially distinguished the groups. Exploratory analyses suggested that the variability, rather than the central tendency, component of $\hat{\delta}$ was the driving force behind these results. **Conclusions**: White matter in young children with autism appears to be abnormally homogeneous, which may reflect poorly organized or differentiated pathways, particularly in the temporal lobe, which is important for social and emotional cognition.

Key words: autism, white matter, diffusion tensor imaging, fractional anisotropy, developmental, brain

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Introduction

Autism is part of a spectrum of developmental disorders characterized by an onset in early childhood and a triad of impairments: 1) deficits in reciprocal social interaction, 2) delayed and/or abnormal language and communication skills, and 3) a narrow range of interests, dependence on routine or ritual, and/or repetitive movements (American Psychiatric Association [DSM-IV-TR], 2000). A complex and heterogeneous disorder, its neural substrates have yet to be elucidated. The most consistent neurobiological finding is an enlargement of brain volume (Piven, Arndt, Bailey, & Andreasen, 1996; Courchesne et al., 2001; Hazlett et al., 2005), which is detectable as early as two years of age, affects both white and gray matter volume, and may be related to decreased axonal pruning early in development (Frith, 2003).

Brain imaging studies have also specifically implicated circumscribed networks in relation to certain symptomatic domains, such as temporal (Aylward et al., 1999; Adolphs, Sears, & Piven, 2001) and fronto-striatal (Sears et al., 1999; Carper & Courchesne, 2005) networks that may underlie social/emotional and ritualistic/repetitive behavior, respectively. Recently, functional connectivity studies have addressed the ability of the nodes of such networks to work in concert, and many have suggested that communication between these nodes may be suboptimal in autism (Noonan, Haist, & Müller, 2009; Minshew & Keller, 2010).

Increased white matter volume and the implication of neural network integrity raised by functional connectivity studies suggest that there may be differences in white matter tracts forming physical connections between brain areas in autism. Diffusion tensor imaging (DTI) indirectly measures the integrity of these tracts via known tissue water diffusion properties within axons. DTI employs a modification in the application of the standard radiofrequency gradients in MRI, rendering it sensitive to tissue water diffusion (Basser & Pierpaoli, 1996). In

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white matter, the myelin sheath and the physical structure of axons impose physical boundaries to the direction of water diffusion, so that diffusion primarily occurs along the axis of the fiber bundles (Beaulieu, 2002), rather than in a freely diffusing state. This directionality is represented by a ratio that describes the diffusion along this primary direction relative to the other two orthogonal directions in three-dimensional space; the most commonly used variant of this ratio is *fractional anisotropy* (FA). At each voxel of the image, FA expresses the deviation of the diffusion from that of spherical or isotropic diffusion, characteristic of what would be seen in freely-diffusing tissue, such as cerebrospinal fluid within the ventricles. Although the relation between FA and underlying histological properties is not fully understood, particularly in areas of crossing or diverging fibers, high FA values generally indicate stronger, more coherently bundled, or more heavily myelinated white matter tracts (Beaulieu, 2002). By unveiling the integrity and coherence of brain white matter representing fiber projections, DTI has the potential to contribute a much richer understanding of white matter structure than conventional MRI alone.

Studies of white matter development in childhood and adolescence as measured by DTI consistently demonstrate that FA increases with age throughout brain white matter (Cascio, Gerig, & Piven, 2007), presumably reflecting ongoing myelination during early childhood and increased organization and coherence of white matter tracts throughout development. DTI may be a particularly promising tool for the study of autism, whose neural underpinnings are likely to involve a distributed network of developing brain systems (Müller, 2007). In a preliminary DTI study of high functioning older children and adolescents with autism to matched controls (Barnea-Goraly et al., 2004), ubiquitous reduction in FA was seen throughout the cerebral cortex using a voxel-based analysis method. More recently, Alexander and colleagues (Alexander et

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al., 2007) demonstrated reduced FA in the corpora callosa of a subgroup of individuals with autism, for whom cognitive abilities were lower than both controls and other autism groups. Keller, Kana, and Just (2007) noted decreased FA in both the corpus callosum and internal capsule of a large sample adolescents and adults with autism, and Lee and colleagues observed decreased FA in the superior temporal gyrus and temporal stem (Lee et al., 2007). In contrast to these studies which focused on older children, adolescents, or adults, a recent study by Ben Bashat and colleagues (2007) found *increased* FA in a small (n=7) group of preschoolers with autism compared to typically developing controls, suggesting a complex developmental trajectory of white matter pathways in autism. This study, however, was limited by a small sample size and a comparison group ranging in age from infants to young adults, in which all members under ten years of age were referred for suspected neurological abnormality.

In the current study, we present DTI data from a large sample of young children with autism compared to a group including typically developing children and children with developmental delay without autism. This sample overlaps with that of a previous study (Hazlett et al., 2005), in which we reported generalized brain volume differences that were particularly robust in white matter. In this report, we also introduce an alternative analysis approach that greatly improves statistical power within a neuroimaging framework and provides a means to summarize the entire distribution of FA values within individual regions of interest (ROIs).

The methods used in this study are based on the principle that, because FA values are bounded by 0 and 1 and are calculated from the covariance of a Gaussian distribution that describes water diffusion, FA itself cannot be Gaussian. Thus, using mean FA directly in a mixed model violates its statistical assumptions, and a viable alternative is to describe FA in terms of its distribution among the voxels in an ROI. The rationale for the use of this method is

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based on first principles of statistics and is described briefly in the Statistical Analysis section. Further detail on the theory and use of this method can be found in the supplementary material, Clement et al. (2008), and Clement-Spychala et al. (2010).

Methods

Sample

A subset of the sample used in our longitudinal study of brain development in autism (Hazlett et al., 2005) was used for this study. In this subset, 33 were diagnosed with autism, 8 with developmental delay without autism (DD), and 17 were typically developing (TD). During the course of the longitudinal study, an upgraded sequence was adopted; the subset included in this report comprises 66 observations from 58 children who were scanned using the newer DTI sequence. A small number in each group had data from two observation points (3 each in the autism and TD groups, 2 in the DD group); these observations were separated by two years' time. We used a hierarchical model to account for the lack of independence between these repeated observations in 8 participants. The participants were recruited and characterized as described previously (Hazlett et al., 2005); all participants in the autism group met ADI-R and ADOS criteria for autistic disorder, and diagnosis was confirmed by the opinion of a licensed clinical psychologist using DSM-UV criteria. Descriptive statistics can be found in Table 1.

Image acquisition and processing

All scans were acquired on a 1.5 T GE Signa Advantage MR scanner. T1-weighted structural images were acquired using a 3D IR prepped SPGR protocol with a 256x256x124 image matrix at 0.78125 x 0.78125 x1.5 mm resolution. DTI images were acquired using 4 repetitions of a 12-direction spin-echo single-shot echo planar imaging (EPI) sequence with a

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128x128x30 image matrix at 1.875 x 1.875 x 3.8 mm resolution (with a 0.4 mm gap) using a b-value of 1000 s/mm^2.

Each DTI slice was screened for motion and other artifacts using custom software that automatically removed slices or shots falling outside predetermined parameters (http://www.ia.unc.edu/dev/download/dtichecker/), including slice-to-slice and average-to-average motion, and to correct for the latter. Subsequently, both the correction of eddy-current based image distortions using mutual information-based unwarping and the calculation of the diffusion tensor elements, eigenvectors and eigenvalues were performed using another custom program (Tensorcalc, Stanford University: http://rsl.stanford.edu/moseley/tensorcalc/). These eigenvectors and values were then used to compute FA maps using a customized program: (http://www.ia.unc.edu/dev/download/fibertracking/index.htm).

The 66 FA maps were then coregistered into the same coordinate system using both affine (scaling and translation) and nonlinear (fluid warping) coregistration. These coregistered FA maps were then combined with an ROI mask comprising a white matter segmentation map and lobe parcellation map created using a semi-automated method (Yushkevich et al., 2006) from a population atlas of all subjects' structural MRI with associated tissue segmentation (Hazlett et al., 2005). The goal of this combination of masks was the isolation of white matter within each of the cortical lobes, and the cerebellum. Because of the lack of clear landmarks to mark the boundary between parietal and occipital lobe, this method yields a parcellation that does not separate these two lobes. This application of white matter mask and lobe parcellation to the individual coregistered FA maps allowed the extraction of FA values for global (whole brain) white matter, white matter in each cortical lobe, and the cerebellum (see Figure 1).

Statistical Analysis

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Raw FA values were transformed to *B* values, and the distribution of these values was used to approximate a beta distribution using two summary parameters estimated from the distribution of the raw *B* values (for details, see supplementary material). The raw FA distributions, transformed to the *B* variable, are overlaid with the approximated beta distributions in Figure S1 for six randomly chosen participants. The plots in supplementary figure S1 illustrate how accurately the transformation represents the raw data, but the bounded nature of the beta distribution resulted in limitations to fit at the boundaries of the distribution (Figure S1A, top-right and bottom-left). Because of this limitation, beta distributions were then transformed to a non-bounded *F* distribution using first principles and a well-known statistical transformation meant to spread the data for statistical analysis. An estimated mean and standard deviation of the approximating *F* were calculated for each distribution. This transformation yields a non-bounded distribution, and unlike the beta value, the *F* value varies in the same direction as FA, which simplifies understanding of the relationship between the outcome variable and the physical property that it represents. A further strength of this conversion is that summary statistics are used to calculate the *F* values rather than individual FA values, resulting in an inherently more reliable and stable variable that is both statistically and scientifically sufficient, as evidenced by how well the distributions fit the real data (Figure S1B). Furthermore, doing so eliminates the high dimension, low sample size problem inherent in imaging studies and allows the use of classical multivariate and longitudinal analyses.

We used the single value δ, representing the mean plus one standard deviation ($\delta = \mu + \sigma$) of the *F*-distribution as our outcome variable. δ and similar robust measures have been used successfully in previous imaging studies (Puff et al., 1994; Clement et al., 2008; Clement-Spychala et al., 2010). In the distributions of interest, δ corresponds to roughly the 85th

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percentile. Because each statistic is based on thousands of FA data values, the beta and equivalent *F* moment estimates can be expected to follow Gaussian distributions rather well (a standard consequence of large-sample theory for maximum likelihood estimates; this assumption is confirmed in Figure S2). Separate general linear mixed models assuming Gaussian distributions of $\hat{\delta}$ were fitted separately for the global white matter, lobe, and cerebellum regions of interest, based on Restricted Maximum Likelihood Estimation (REML). Group, age, and gender served as between-participant predictors for the analysis of all ROIs. For the lobe and cerebellum models, side (hemisphere) was also included as a within-participant predictor, as was lobe in the lobe model. The age and gender variables were centered about 4 years and 0.8 respectively, the approximate overall mean age and proportion of males.

Our primary planned analysis consisted of three separate models with $\hat{\delta}$ as the outcome variable for global white matter, individual cerebral lobes, and the cerebellum. Further planned analyses included tests of group by lobe interactions, regardless of the outcome of the main lobes model. In secondary analyses, we explored the contributions of slice thickness and cognitive development scores. Finally, a secondary analysis that explored the relative contributions of the components of δ (μ and σ) was performed.

Results

Primary Analysis

Results for global white matter

For global white matter, the model revealed a statistically significant effect of group ($F(2,54) = 7.77$, $p = 0.0011$), with the autism group having a significantly lower $\hat{\delta}$ than the combined control group ($t(54) = -3.8$, $p = 0.0003$). This difference seems to be driven by a significant difference between the autism and typically-developing (TD) groups ($t(54) = -3.8$, p =

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0.0003), although the autism group also differed significantly from the DD group ($t(54) = -2.3$, $p = 0.0254$). As expected, a significant effect of age ($F(1,54) = 39.7$, $p < 0.0001$) was also observed. In Figure 2, predicted change with age in each of the three groups is illustrated. The typical group shows the steepest linear increase in predicted $\hat{\delta}$ with age, while the DD group's slope is shallowest; however there was no significant interaction between age and group. The model output is summarized in Table 2.

Results for specific lobes within the white matter mask

The linear mixed model (summarized in Table 3) for the lobe analysis revealed that lobe ($F(2, 110) = 92.04$, $p < 0.001$), group ($F(2,54) = 6.72$, $p = 0.0025$), side (hemisphere) ($F(1,55) = 145.02$, $p < 0.0001$) and age ($F(1,320) = 21.74$, $p < 0.0001$) were significant predictors of $\hat{\delta}$., while a test for interaction between age and group was nearly significant ($F(2,320) = 2.96$, $p=0.0532$) at a significance level of 0.05. Follow-up analyses revealed significant differences between the autism and combined control group ($t(54) = -2.99$, $p = 0.0042$), reflected in significant differences between both the autism and TD groups ($t(54) = -2.12$, $p = 0.0042$), and between the autism and DD groups ($t(54) = -3.48$, $p = 0.0010$); the overall lower predicted values of $\hat{\delta}$ across lobe and hemisphere in the autism group compared to both control groups is depicted in Figure 3. A statistically significant interaction between group and age for the DD vs. typical groups ($t(320) = 2.38$, $p = 0.0181$) was also observed, but other group by age interactions did not reach significance.

Although there was no overall statistically significant interaction between group and lobe, several significant relationships emerged when the groups were compared with pre-planned t-tests. The autism and combined control groups differed significantly in the $\hat{\delta}$ value for all three

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lobes, as summarized in Table 4. However, when a Bonferroni correction was used to correct for multiple tests, only the group differences in the temporal lobe remained.

Results for cerebellum within the white matter mask

The linear mixed model for the cerebellum analysis yielded significant effects of group ($F(2,54) = 12.38$, $p < 0.0001$), side ($F(1,54) = 63.74$, $p < 0.0001$), and age ($F(1,54) = .16$, $p < 0.0001$) in $\hat{\delta}$. When analyzing the autism group compared to the control groups separately, the autism group differed significantly from the combined control group ($t(54) = -4.95$, $p = 0.0010$), as well as the separate TD ($t(54) = -3.93$, $p = 0.0020$) and DD ($t(54) = -3.95$, $p = 0.0020$) groups. The results of the model are summarized in Table 5. No significant interactions between group and age were found; the predicted $\hat{\delta}$ values across lobe and hemisphere as a function of age are illustrated in Figure 4. As in the previous models, the autism and typical groups follow similar trajectories, but the autism group has a much lower level of predicted $\hat{\delta}$, while the DD group has a static level of predicted $\hat{\delta}$ throughout the five-year span.

Secondary Exploratory Analyses

Alternative outcome measures

A separate multivariate model was run using each of the two components of $\hat{\delta}$ (F-mean and F-standard deviation from the approximated F distribution) as outcome variables separately, revealing that group differences were driven almost entirely by the standard deviation component. Follow-up group tests for the standard deviation model yielded significant differences for the autism versus TD ($t = -4.07$, $p = 0.002$) and autism versus DD ($t = -3.18$, $p = 0.0018$) groups, but no significant differences for the TD versus DD group, using F-standard deviation (F-SD) as the outcome variable. No significant differences in any group comparisons

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were found using the *F*-mean as the outcome variable. The *F*-SD outcome variable is a reflection of the *variability* of FA values within the distribution. The exploratory results suggest that the autism group has similar overall FA levels within an ROI, but less variation in FA compared to the other two groups.

Importantly, we also explored the non-transformed FA distribution properties as outcome measures, which resulted in a substantial loss of power, similar to the loss of power that might be observed in simply taking the mean FA value. The transformation to an *F*-distribution is based completely on the first principles of statistics and allows for the summarization of the entire data set, effectively eliminating the high dimension, low sample size problem, which provides greater power to detect specific differences.

Additional predictors

Because the groups' mean estimated IQs differ, we ran the global model with cognitive development score included as a predictor. No significant main effects or interactions between cognitive development score and group were found to predict δ. This provides validation for the consideration of the two control groups as a combined group, although results are also presented for the groups separately.

Although a nominal value of 4 mm was specified for the z-direction of the diffusion-weighted images, we found upon inspection of the data that thickness actually varied between 4.09 and 4.21 mm. This variability was wider than expected, and the source of it was unknown. As a result, each model was also run with slice thickness included as a covariate. Thickness did have a significant effect on the outcome measure δ ($F(1,54)=9.77$, $p=0.0029$), but the significant differences reported in the above section, as well as the predicted trajectories with age, were not altered by the addition of this covariate. Thus, we are confident that the variability in slice

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thickness does not represent a confound that affects the group differences highlighted in this report but wish to point out that potential variability in slice thickness is a factor that future experiments should take into account.

Discussion

This study employed a novel approach for the analysis of diffusion data, focusing on FA distributions rather than mean FA values in the brain regions of interest. Using this technique, we noted significant group differences in cerebral and cerebellar white matter. The autism group had significantly lower δ values than the combined control group, both globally, and within specific cortical lobes, as well as in the cerebellum. Although the longitudinal data in this study were not complete, the qualitative predicted trajectories of δ between ages 1.5 and 6.5 years followed group-specific patterns, with the DD group exhibiting a flat trajectory and the autism group increasing in a manner more similar to the typical group, despite its lower overall δ values.

The statistical approach we employed is an alternative that addresses the high dimension, low sample size problem inherent in neuroimaging, reducing thousands of voxels accurately to two summary parameters via transformation of the FA values into standard distributions with well-known statistical properties. This analysis method provides considerable sensitivity to detect group differences (Clement-Spychala et al., 2010). In the analysis, we considered *F*-distribution means, the SDs, and the combination of these values ($\delta = \mu + \sigma$) as potential outcome measures. While our primary analysis of δ revealed significant group differences, closer inspection revealed that these differences were driven almost entirely by the standard deviation component of δ. Therefore, the standard deviation (*F*-SD) was considered as the main outcome variable for secondary analyses. The exploratory results suggest that the *variability* of FA values within the distributions was the key factor in distinguishing the groups. This

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diminished variability in FA may reflect a decrease in the differentiation that typically occurs during brain maturation. Our results are thus consistent with other DTI studies employing more traditional analysis approaches and finding abnormal developmental trajectories of white matter maturation (Ben-Bashat et al., 2007), while providing more power and sensitivity to detect differences and employing more established and well-founded statistical properties. The transformations we applied to the FA values do not change the biological significance of the outcome variable, rather they allow us to describe FA more comprehensively within a circumscribed region of the brain than would be possible with only a mean FA value.

The data in Table 3 and Figure 3 suggest that there are different patterns over time that partially distinguish the groups. A nearly significant interaction between age and group was observed ($p=0.0532$), with significant contrasts in the TD vs. DD comparison of group by age. Although the small size of the DD group prevents firm conclusions, the impression from these tests and Figure 3 is that the trajectory is steepest in the TD group, and shallowest in the DD group, while that of the autism ASD group is intermediate between them.

The lobe analysis also revealed that group differences in frontal, temporal, and occipital-parietal lobes distinguished the autism group from the control groups, both separately and in combination, as evidenced by main effects of group in Table 3 and the pre-planned t tests displayed in Table 4. Both the temporal and frontal lobes have been heavily implicated in autism: the temporal lobe being the hub of a wide neural network mediating social and emotional processes (Schultz et al., 2000; Amaral & Corbett, 2003; Boddeart et al. 2004; Mosconi, Mack, McCarthy, & Pelphrey, 2005), and the frontal lobe a key structure in executive abilities such as set shifting and cognitive flexibility (Turner, 1999; Schmitz et al., 2006; Shafritz, Dichter, Baranek, & Belger, 2008). Although multiple interpretations of reduced FA variability are

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plausible, one possibility is that it reflects early disruption of widespread cortical networks in autism, which is consistent with a growing literature emphasizing autism as a cortical disconnection syndrome (Geshwind & Levitt, 2007; Minshew & Williams, 2007). Occipital and parietal cortex, as well as superior temporal sulcus, a site of group differences in the Lee study (2007), are largely devoted to processing sensory information, and the evidence of their disruption early in autism suggests that sensory processing deficits should be more closely examined in autism. However, after correcting for multiple comparisons, the only group differences that remained were in the temporal lobe, consistent with many other studies demonstrating that social cognition mediated by the temporal lobe is among the most fundamental deficits in ASD.

We also noted group differences in the cerebellum, with the autism group again displaying significantly lower $\hat{\delta}$ than both control groups. A similar pattern was also observed when predicting $\hat{\delta}$ over time; the autism group did increase predicted δ with age, but overall $\hat{\delta}$ was greatly decreased relative to both control groups. Reported volumetric differences of the cerebellum in autism are proportional to overall differences in brain volume (Sparks et al., 2002; Palmen et al., 2005). While cerebellar white matter increase has been reported in young children with autism (Courchesne et al., 2001), we did not observe this in a recent study of a larger sample of two year-olds (Hazlett et al., 2005). In spite of this, we find significant differences in the FA distribution in the cerebellum in these same young children, suggesting that the early stages of cerebellar white matter organization into fiber pathways is somehow altered in autism, without affecting overall white matter volume in the cerebellum.

To our knowledge, this is the first report of the properties of FA distributions in young children with autism. The abnormal $\hat{\delta}$ values, driven by the standard deviation of the

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transformed *F*-distributions, may reflect more homogeneous white matter in autism, typical of what would be expected in younger children. Although the use of a novel statistical method was a unique advantage, the study was limited by the large neuroanatomical regions from which the FA values were extracted. Current research in our group focuses on new methods for population-based statistics of individual fiber tracts (Goodlett et al., 2006), segmented from high-resolution DTI on 3 Tesla scanners.

The variability in image slice thickness was another limitation intrinsic to the imaging hardware and/or acquisition software. This variable did not affect any group differentially, thus our findings are not compromised, but this is a potential confounding variable that future studies should take steps to monitor.

An important finding was the patterns predicted trajectories of the three groups throughout the age range of the sample (two to six years). In the lobes analysis, a small group of children with nonspecific developmental delay (DD) maintained a steady level of $\hat{\delta}$ relative to controls throughout the age range. As can be seen from Figures 3, this translates to an abnormally high level of $\hat{\delta}$ in DD relative to the typical group until about age three or four, at which point the typical control group, which starts with lower $\hat{\delta}$, but increases steadily, catches up and surpasses the DD sample. It is important to note, however, that although the overall interaction between group and age was significant for the lobes analysis, it was not for the global analysis, and that contrasts among the three groups only showed significant interactions between group and age for the typical and DD groups. It is possible that increased heterogeneity in the autism group limited statistical power to detect the difference from the DD group that is suggested by Figures 2-4. Our preliminary analyses separating the components of $\hat{\delta}$ suggest that these differences reflect the predicted individual variability in FA among the groups. This

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indicates that an autism-specific reduction of variability in white matter diffusion, beyond what is seen in general delay, is present in the first five years of life. In addition, although the size of the DD group limits definitive conclusions, the flat slope in the DD group is consistent with a longitudinal study by Shaw and colleagues (2006) in which the trajectory of change in cortical thickness was associated with generalized intellectual ability, suggesting that early brain plasticity is closely related to cognitive development. This early window is a critical time in neural development, and the impact of early white matter organization is fundamental to properly functioning neural networks that process information and allow behavioral adaptation throughout the lifespan. The finding of group differences in the variability of transformed diffusion parameters is also consistent with a recent paper (Johnson, 2011) suggesting that the brain basis for developmental disabilities could be conceptualized as delayed specialization (maintaining more generalized patterns and processes longer before developing more targeted or specific ones).

Future studies should focus on: 1) more comprehensive exploration of the variability in FA distributions, 2) longitudinally following this and other samples to determine how the trajectories change in later childhood and adolescence, 3) combining DTI measures in more circumscribed areas, e.g., in well defined fiber tracts of interest associated with specific brain function, with functional imaging to elucidate the most important neural networks affected in autism, 4) replication with a larger idiopathic DD group, and 5) relating the dysfunction in these systems to the complement of behavioral symptoms that characterize autism.

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Financial Disclosures

None of the authors have conflicts of interest to declare.

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Running head: Cascio 2-6 Year-Old Autism FA Distributions

Table and Figure Legends

Table 1. Summary demographic data on the sample. Age is the mean for all observations in the group. TD: typically-developing; DD: developmentally delayed. *Estimated IQ, based on measures of cognitive development (Vineland Adaptive Behavior Scale, from which the communication domain was used to compute verbal IQ (VIQ) and Mullen Scales of Early Learning, from which the visual reception and fine motor subtests were used to compute performance IQ (PIQ). ADI: Autism Diagnostic Interview (Social composite cutoff for autism – 10); ADOS: Autism Diagnostic Observation Schedule.

Table 2. Tests of whole brain white matter (global) main effects model, with $\hat{\delta}$ as the outcome variable. Num DF: numerator degrees of freedom, Den DF: denominator degrees of freedom.

Table 3. Tests of lobes main effects model, with $\hat{\delta}$ as the outcome variable. Num DF: numerator degrees of freedom, Den DF: denominator degrees of freedom.

Table 4. Group comparisons $\hat{\delta}$ of by lobe. SE: Standard error. * Denotes significant t test that survived a Bonferonni correction (p = 0.05/12=0.004).

Table 5. Tests of cerebellum main effects model, with $\hat{\delta}$ as the outcome variable. Num DF: numerator degrees of freedom, Den DF: denominator degrees of freedom.

Figure 1. Creation of cortical ROIs. Lower left panel (C). cortical lobe parcellation map. Panels A, B, and D: axial, sagittal, and coronal views of the thresholded white matter mask superimposed on the cortical segmentation map.

Figure 2. Predicted global δ as a function of age by group. Data points from the same individual at different time points are connected by dashed lines.

Figure 3. Predicted lobe δ as a function of age by group. Data points from the same individual at different time points are connected by dashed lines. Data points represent mean values across lobe and hemisphere.

Figure 4. Predicted cerebellar δ as a function of age by group. Data points from the same individual at different time points are connected by dashed lines. Data points represent mean values across lobe and hemisphere.

Key Points

- Diffusion tensor imaging studies of children with autism spectrum disorders (ASD) support decreased fractional anisotropy (FA) of white matter tracts.

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Running head: Cascio 2-6 Year-Old Autism FA Distributions

- Previous studies have been limited by small samples, older children, and analysis techniques with limited power.
- The current study improved power by focusing on the distribution of individual FA values in a large sample of very young children with autism.
- A parameter summarizing the mean and standard deviation of a statistically based transformation of the FA distribution was significantly decreased in autism; the standard deviation drove the group difference.
- Predicted developmental trajectories of this parameter across the age range in the sample distinguished the DD from the typical group.
- These results may reflect poorly organized or differentiated neural pathways in children with ASD and altered developmental trajectories for children with DD.

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Table 1

	Autism	TD	DD	Total
N participants	33	17	8	58
N observations	36	20	10	66
IQ* [Mean, (SD)]	57.3 (17.3)	110.5 (18)	58.9 (10.1)	73.1 (29.3)
VIQ	53.9 (12.9)	95.9 (10.4)	64.2 (13.7)	
PIQ	25.6 (10.1)	54.9 (8.6)	28.7 (8.2)	
Age [Mean Years, (SD)]	4.6 (1.1)	3.2 (1.3)	3.93 (1.1)	4.1 (1.3)
% Male	91.7	80	50	81.8
ADI Social Composite	20.11 (5.9)	-	-	
ADOS Communication + Social Interaction	17.2 (3.3)	-	-	

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Table 2

Effect	Num DF	Den DF	F	p	Contrast	DF	t	p
Group	2	54	7.77	0.0011	Group: *Autism vs Combined*	54	-3.8	0.0003
Gender	1	54	1.00	0.3217	Group: *Autism vs TD*	54	-3.8	0.0003
Age	1	54	39.7	<0.0001	Group: *Autism vs DD*	54	-2.3	0.0254
Age*Group	2	54	2.10	0.1318	Group: *TD vs DD*	54	-0.5	0.6436
					Group*Age: *Autism vs Combined*	54	-0.7	0.5023
					Group*Age: *Autism vs TD*	54	-1.5	0.1512
					Group*Age: *Autism vs DD*	54	0.79	0.4356
					Group*Age: *TD vs DD*	54	1.89	0.0641

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Table 3

Effect	Num DF	Den DF	*F*	p	Contrast	DF	*t*	p
Group	2	54	6.72	***0.0025***	**Group:** A*utism vs Combined Control*	54	-2.99	***0.0042***
Lobe	2	110	92.04	***<.0001***	**Group:** A*utism vs TD*	54	-2.12	***0.0384***
Side	1	55	145.02	***<.0001***	**Group:** A*utism vs DD*	54	-3.48	***0.0010***
Gender	1	54	0.84	0.3632	**Group:** *TD vs DD*	54	0.65	0.5163
Age	1	320	21.74	***<.0001***	**Group*Age:** A*utism vs Combined Control*	320	0.21	0.8354
Group*Lobe	4	110	1.31	0.2713	**Group*Age:** A*utism vs TD*	320	0.90	0.3667
Group*Side	2	55	0.37	0.6942	**Group*Age:** A*utism vs DD*	320	1.54	0.1242
Age*Group	2	320	2.96	0.0532	**Group*Age:** *TD vs DD*	320	2.38	***0.0181***
Lobe*Side	2	110	63.55	***<.0001***				
Group*Lobe* Side	4	110	0.67	0.6123				

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Table 4

Lobe	*Group 1*	*Group 2*	*Difference*	*SE*	*DF*	*t*	*p*
Frontal	*Autism*	*Combined Control*	-0.0403	0.019	110	-2.14	***0.0342***
	Autism	*TD*	-0.0302	0.023	110	-1.31	0.1937
	Autism	*DD*	-0.0603	0.022	110	-2.78	***0.0064***
	TD	*DD*	-0.0301	0.027	110	-1.12	0.2651
Occipital/Parietal	*Autism*	*Combined Control*	-0.0568	0.020	110	-2.82	***0.0057***
	Autism	*TD*	-0.0553	0.026	110	-2.16	***0.0333***
	Autism	*DD*	-0.0597	0.021	110	-2.88	***0.0048***
	TD	*DD*	-0.0044	0.028	110	-0.15	0.8722
Temporal	*Autism*	*Combined Control*	-0.0655	0.019	110	-3.46	***0.0008****
	Autism	*TD*	-0.0615	0.024	110	-2.51	***0.0136***
	Autism	*DD*	-0.0731	0.018	110	-4.18	***<.0001****
	TD	*DD*	-0.0116	0.025	110	-0.58	0.5621

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Table 5

Effect	Num DF	Den DF	*F*	p	Contrast	DF	*t*	p
Group	2	54	12.38	***<.0001***	**Group:** A*utism vs Combined Control*	54	-4.95	***<0.001***
Side	1	54	63.74	***<.0001***	**Group:** A*utism vs TD*	54	-3.93	***0.0002***
Gender	1	54	1.47	0.2303	**Group:** A*utism vs DD*	54	-3.95	***0.0002***
Age	1	54	28.16	***<.0001***	**Group:** *TD vs DD*	54	-1.43	0.1574
Group*Side	2	54	0.12	0.8904	**Group*Age:** A*utism vs Combined Control*	54	1.57	0.1234
Age*Group	2	54	1.54	0.2241	**Group*Age:** A*utism vs TD*	54	0.82	0.4163
					Group*Age: A*utism vs DD*	54	1.70	0.0951
					Group*Age: *TD vs DD*	54	1.20	0.2368

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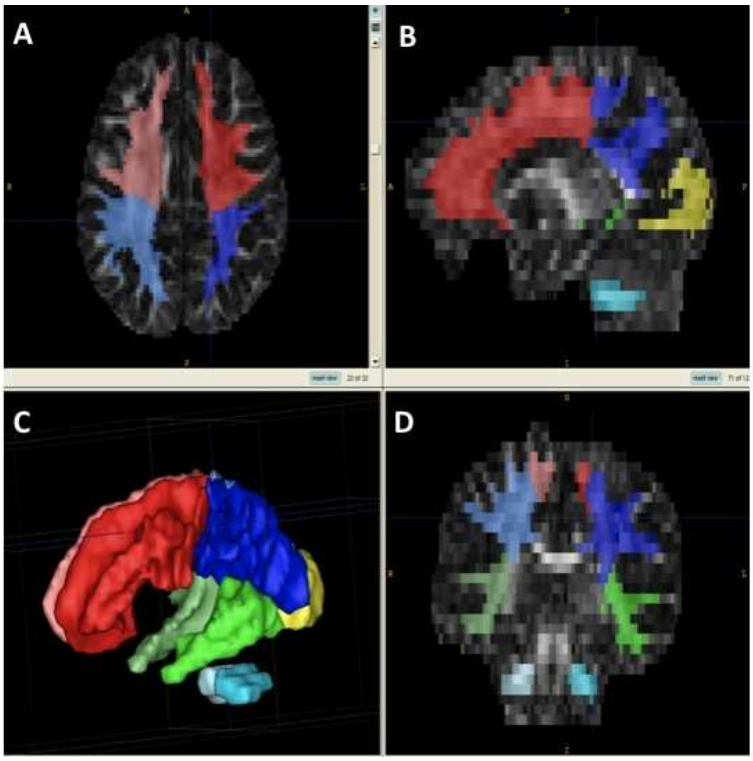

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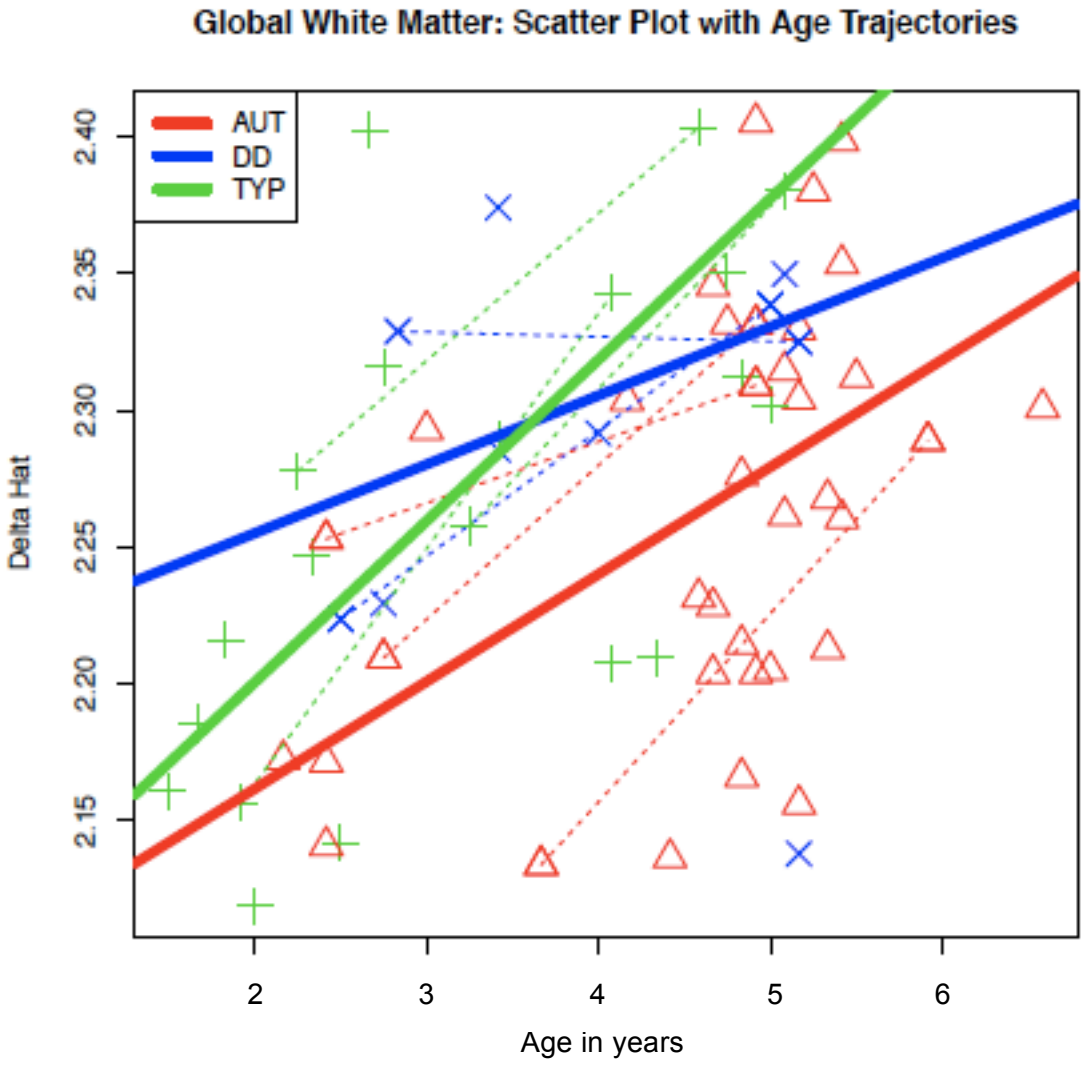
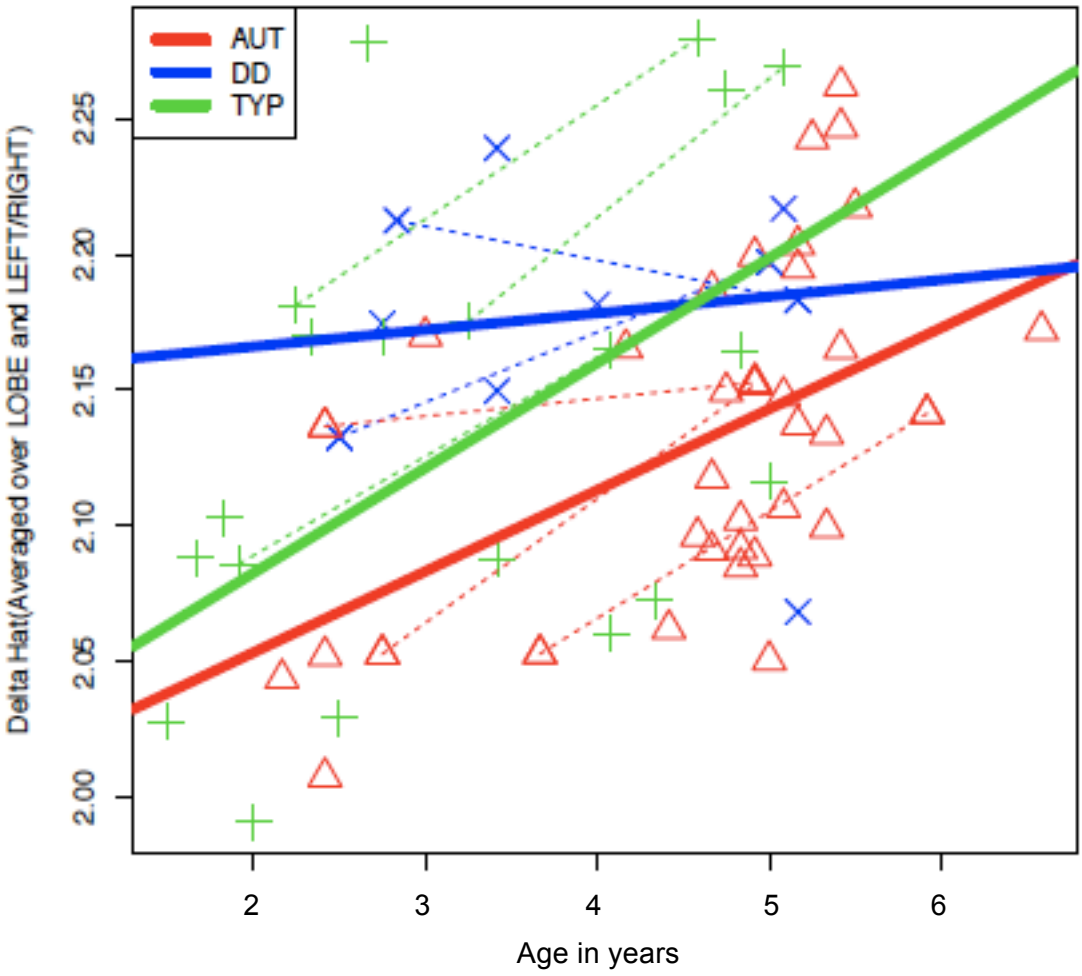
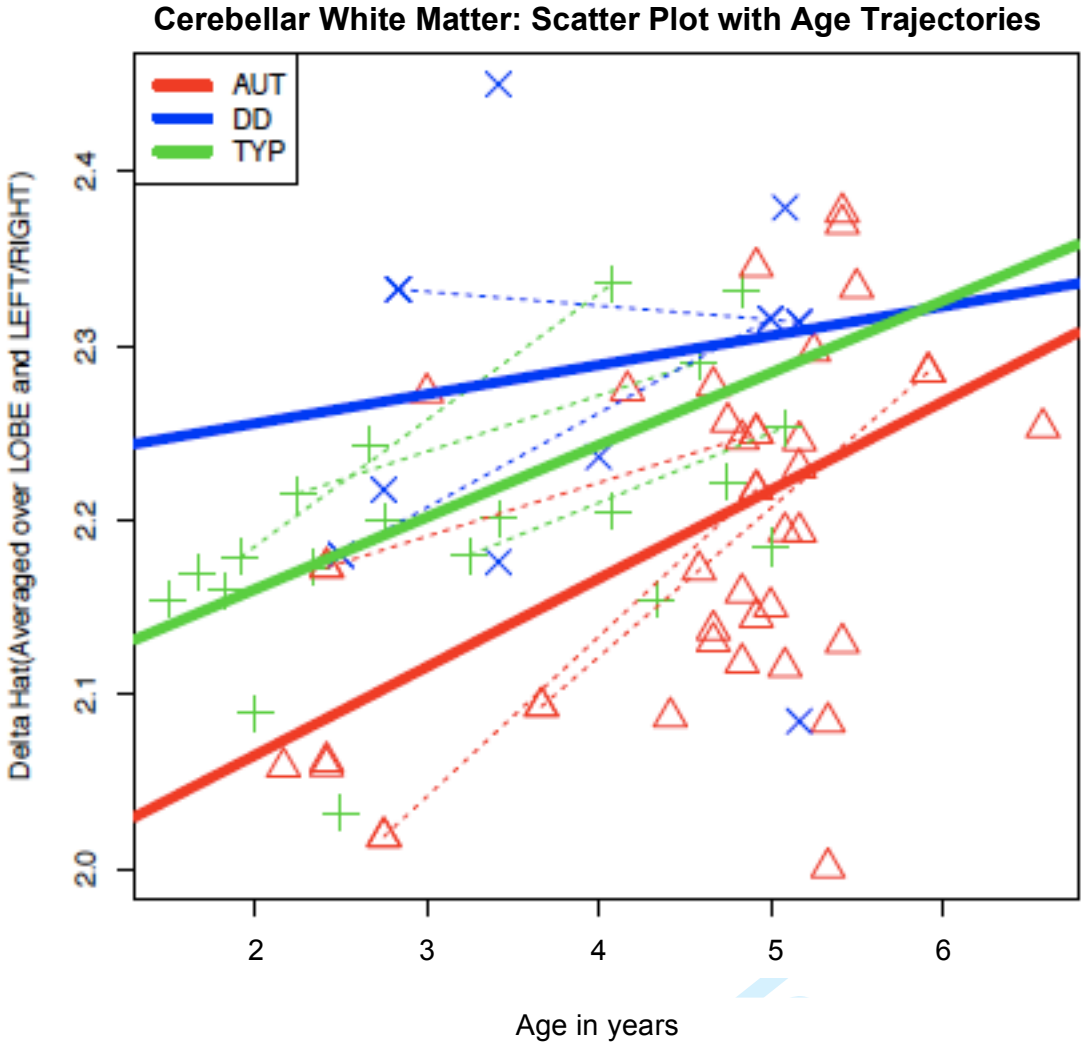

Global White Matter: Scatter Plot with Age Trajectories
AUT
DD
TYP
Delta Hat
2.15
2.20
2.25
2.30
2.35
2.40
2
3
4
5
6
Age in years


Lobes White Matter: Scatter Plot with Age Trajectories
AUT
DD
TYP
Delta Hat(Averaged over LOBE and LEFT/RIGHT)
2.00
2.05
2.10
2.15
2.20
2.25
2
3
4
5
6
Age in years


Cerebellar White Matter: Scatter Plot with Age Trajectories
AUT
DD
TYP
Delta Hat(Averaged over LOBE and LEFT/RIGHT)
2.0
2.1
2.2
2.3
2.4
2
3
4
5
6
Age in years

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Supplementary Material

Details on theory and implementation of statistical analysis

Background and transformation to beta distribution

Diffusion imaging measures of the movement of water molecules, which depends on Brownian motion. Brownian motion may be described as a Gaussian distribution from which covariance matrices are calculated to obtain FA. These covariances are distributed as a type of multivariate Chi-Square (called a Wishart). First principles of fundamental statistical theory say that FA, a bounded variable (with values between 0 and 1) that is a function of those covariances, is not Gaussian. Instead, FA is a function of Wisharts. This knowledge disallows one from using a strict FA value in standard statistical models such as the mixed model described below. Thus we describe and analyze FA within large ROIs not as a mean, but as a distribution of values. The rationale for the use of this method is based on first principles of statistics and is described briefly in the Statistical Analysis section. Further detail on the theory and use of this method can be found in Clement et al. (21-22).

The FA value is bounded by 0 and 1 and measures the deviance from sphericity, (i.e. the degree to which the diffusion deviates from equality in all three directions of three-dimensional space). Hence we transformed FA to a widely used statistical measure of sphericity, $\hat{\varepsilon} = 1 - (2/3)\mathrm{FA}^2$. As FA increasesand diffusion becomes more anisotropic, $\hat{\varepsilon}$ decreases.

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The maximum likelihood estimate (MLE) of the parameter $\varepsilon, \hat{\varepsilon}$, is a one-to-one function of the locally best invariant (LBI) test statistic for sphericity, thus resulting in

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maximized power to detect group differences. A region of interest with many voxels generates a frequency distribution of $\hat{\varepsilon}$ values. The frequency distribution of a simple function of $\hat{\varepsilon}$, namely $B = \sqrt{(3/2) * \hat{\varepsilon} - 1/2} = \sqrt{1 - FA^2}$, can be approximated well by fitting a beta distribution, which is defined by two summary parameters, α and γ. This process accurately summarizes and characterizes a large number of FA values with two parameters for each distribution of values for an ROI, eliminating the high dimension, low sample size problem inherent in imaging studies.

Transformation from beta to *F* distribution

The additional transformation from Beta to *F* is based on first principles of statistics, maintains a good fit with the transformed FA values (Figure 1b), and is accomplished by the following formula: $R = \frac{(1-B)/\gamma}{B/\alpha} \sim F(2\gamma, 2\alpha)$, where α and γ are summary statistics for the Beta distribution, functions of which are analogous to μ and σ in the normal distribution. This approachtakes good advantage of inherent distributional properties to improve sensitivity to group differences relative to the standard approach of reporting mean FA values in a small region of interest. Appropriate statistical analyses targeted to the non-Gaussian shape of the FA distribution can be expected to increase power relative to simply analyzing the mean observed FA value, which fails to account for the change in shape among FA distributions for various participants, and also seems likely to be inappropriately sensitive to outliers and imaging artifacts.

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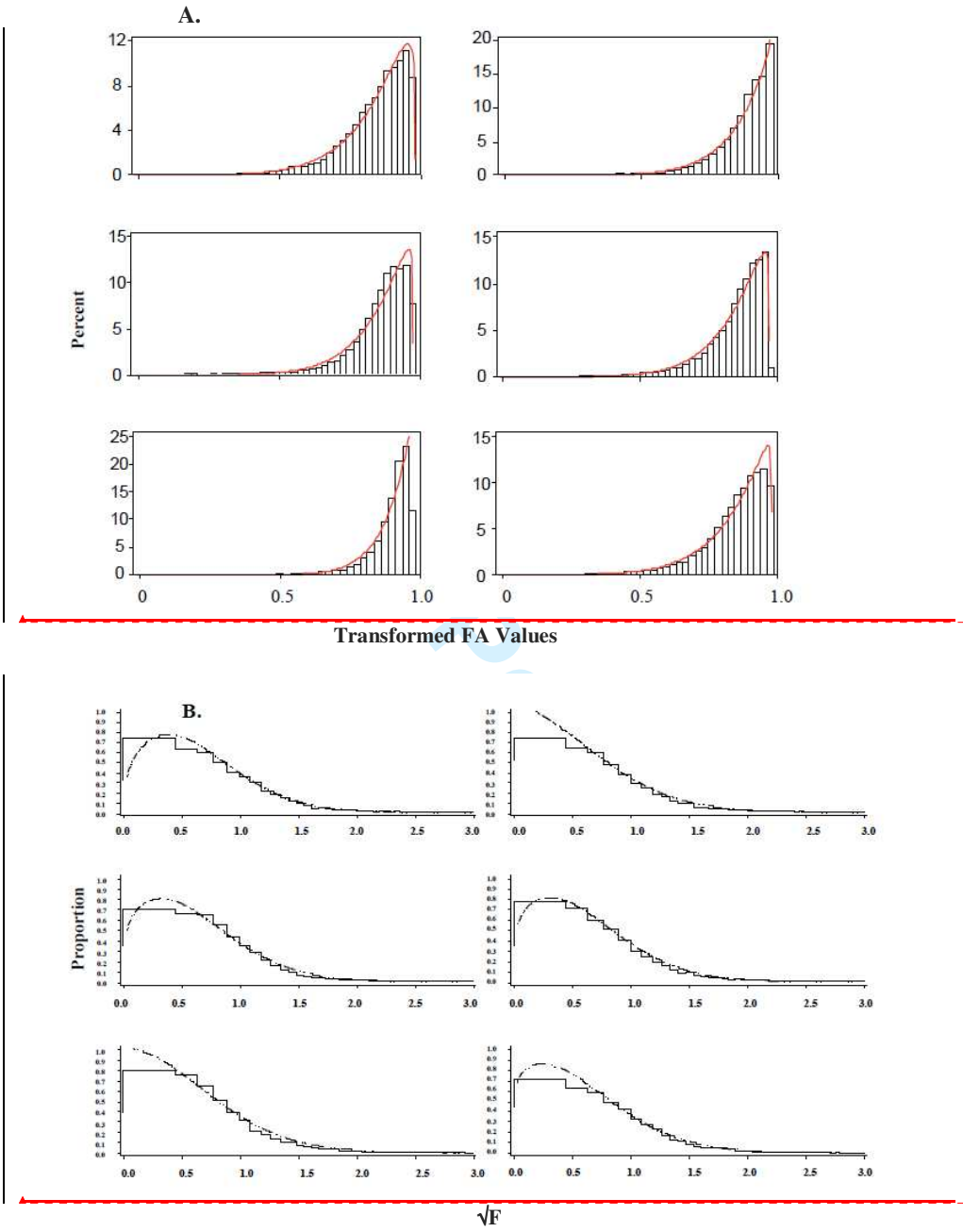
Figure S1



A: Plots illustrating the fits of the Beta distributions to the transformed global FA distributions for six randomly chosen subjects. B: The F-distribution fit to transformed global FA values for the same six subjects.

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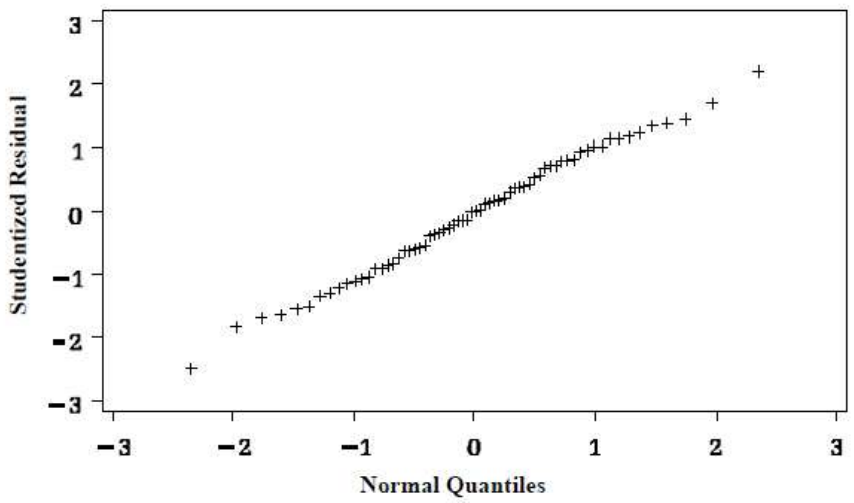
Figure S2.



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Residuals for the global model plotted against quantiles of a normal distribution.

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