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A Longitudinal Study of White Matter Development in Relation to Changes in Autism Severity Across Early Childhood

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Abstract

Background: Cross-sectional diffusion-weighted MRI studies suggest that young autistic children have alterations in white matter structure that differ from older autistic individuals. However, it is unclear if these differences result from atypical neurodevelopment or sampling differences between young and older cohorts. Furthermore, the relationship between altered white matter development and longitudinal changes in autism symptoms is unknown.

Methods: Using longitudinal diffusion-weighted MRI acquired over 2-3 timepoints between the ages of approximately 2.5-7 years in 125 children with autism and 69 typically developing controls, we directly tested the hypothesis that individuals with autism have atypical white matter development across childhood. Additionally, we sought to determine whether changes in white matter diffusion parameters were associated with longitudinal changes in autism severity.

Results: Children with autism were found to have slower development of fractional anisotropy (FA) in the cingulum bundle, superior longitudinal fasciculus, internal capsule, and splenium of the corpus callosum. Furthermore, in the sagittal stratum, autistic individuals who increased in severity over time had a slower developmental trajectory of FA compared to individuals who decreased in severity. In the uncinate fasciculus individuals who decreased in severity also had greater increases in FA with age.

Conclusions: These longitudinal findings indicate that previously reported differences in diffusion-weighted MRI measures between younger and older autism cohorts are attributable to an

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atypical developmental trajectory of white matter. Differences in white matter development between individuals who increased, remained stable, or decreased in autism severity suggest that these functional differences are associated with fiber development in the autistic brain.

Keywords

imaging; autism; longitudinal; diffusion; white matter; development

Introduction

Autism spectrum disorder (ASD) is an etiologically and phenotypically heterogenous neurodevelopmental condition characterized by core deficits in social communication and restricted, repetitive behaviors (1). It has been theorized that ASD symptoms are associated with atypical neural connectivity (2). Diffusion weighted MRI (DW-MRI) has been a valuable method to study structural properties of white matter in ASD. A growing body of literature suggests that young children and older individuals with ASD show opposite relationships between DW-MRI measures of white matter microstructure when compared to typical development (TD) (3–11). However, longitudinal studies needed to confirm this atypical white matter development in autism are limited (6,8,12,13). Furthermore, the relationships between altered white matter development and longitudinal changes in autism symptoms are unclear.

Of current available methods, DW-MRI represents one of the best ways to investigate microstructural properties of white matter in-vivo. Diffusion tensor-based metrics, including fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AD), measure structural variance attributable to several underlying axonal properties including density and diameter, orientation, tortuosity, membrane permeability, and myelin content (14). While white matter tracts studied and implicated in autism vary to some degree from study to study, the directionality of DW-MRI results is generally consistent. Studies of older children, adolescents and adults report decreased measures of FA in autism (15,16), with comparatively few exceptions (17,18). In contrast, a smaller body of literature reports increases in FA in autism during early childhood (3–10). Longitudinal studies are needed to determine if differences between cross-sectional DW-MRI findings in early childhood and older ASD samples are related to altered white matter development or are attributable to methodological artefacts (e.g. cross-sectional participant sampling differences, head motion).

To our knowledge, only four studies have longitudinally evaluated white matter development in young children with ASD. One study of high-risk infant siblings found that infants who were later diagnosed with ASD had both higher FA values at 6 months and slower development of FA from 6-24 months compared to ASD negative infants (6). Another longitudinal study of 12-48 month old toddlers reported a slower developmental course of FA in ASD compared to TD (8). This trajectory was characterized by higher FA values in the youngest autistic children with an apparent inflection around 30-40 months leading to comparative decreases in FA by 3-4 years of age. Two additional DW-MRI studies reported on an accelerated longitudinal sample that covered a large age range (3-41 years) (12,13).

One of these studies reported significant differences in the developmental trajectory of FA within the corpus callosum marked by atypically high FA values among young autistic children that decreased with age, intersecting the TD trajectory around 7 years of age before plateauing and resulting in decreases in FA compared to TD across adolescences and adulthood (12). The other study characterized the longitudinal development of the internal capsule and reported early decreases in FA in ASD that later "catch up" to TD cases with age (13). Thus, collectively these studies provide some longitudinal evidence that young children with ASD have both early increases in and slower development of FA measures compared to TD (8,12), but are not fully consistent (13).

Beyond clarifying differences in white matter development between ASD and TD, a critical question to resolve is whether altered white matter development is associated with significant clinical differences across subsets of individuals with ASD. While reports find that a majority of autistic individuals have a stable level of severity over time, significant numbers of individuals are reported to either decrease (improve) or increase (worsen) in autism symptom severity across childhood (19). For example, we recently reported that while 54.4% of children with ASD have a stable level of severity from approximately 2.5-7 years of age, 28.8% decrease and 16.8% increase significantly over this period (20).

In the current study, we sought to characterize the developmental trajectory of white matter structure in ASD compared to TD from approximately 2.5-7 years of age. We utilized longitudinal DW-MRI acquired during natural nocturnal sleep at 2-3 timepoints and linear mixed effects modeling to evaluate longitudinal measures of FA, MD, RD and AD within several white matter tracts previously indicated in ASD. Our primary aim was to test the hypothesis that young children with ASD have altered white matter neurodevelopment characterized by increased FA during early life and a slower developmental trajectory of FA resulting in decreased FA compared to TD at older ages. A secondary aim was to investigate the relationship of white matter development with baseline autism severity and between three defined groups of autism severity change (i.e. increasing, stable, and decreasing severity) across early childhood.

Methods and Materials

Participants

This study included 125 individuals with autism and 69 TD controls (Figure 1, Table 1). Participants were enrolled in either the ongoing UC Davis Medical Investigation of Neurodevelopmental Disorders Institute Autism Phenome Project (APP) or Girls with Autism: Imaging of Neurodevelopment (GAIN) studies. The longitudinal design of these studies includes MRI scanning at three timepoints; enrollment/baseline at 24-42 months of age and annual follow up for two additional time points. All participants were required to be native English speakers, ambulatory, have no contraindications for MRI, no suspected vision or hearing problems or known genetic disorders or neurological conditions. An ASD diagnosis was confirmed by research reliable, trained clinical psychologists using the Autism Diagnostic Observation Schedule-Generic (ADOS-G) (21) or ADOS-2 (22), the Autism Diagnostic Interview-Revised (ADI-R) (23) and DSM-IV-TR criteria (1). ADOS calibrated severity scores were calculated to allow comparison of autism severity across

participants tested with different ADOS modules (19). The Mullen Scales of Early Learning (MSEL) (24) were used to assess developmental quotient (DQ) at enrollment. TD children were excluded if they did not fall within two standard deviations on the MSEL. The current study included all individuals in the APP/GAIN cohorts who had successfully completed structural and diffusion weighted MRI scans with sufficient quality at two or three timepoints. Previous DW-MRI studies have utilized subgroups of the currently described sample (10,25,26). All aspects of the study protocol were approved by the University of California Davis Institutional Review Board. For additional details of sample ascertainment, inclusion criteria, and demographics see supplementary methods and Table S1.

Image Acquisition, Preprocessing, and Region of Interest Approach

All DW-MRI scans were acquired at the Imaging Research Center, UC Davis, Sacramento using a 3T Siemens Total Imaging Matrix (TIM) Trio MR system (Erlangen, Germany). MRI scanning was performed during natural nocturnal sleep during participant's usual bedtime hours without sedation (27). Diffusion weighted images were preprocessed with MRtrix3 (www.mrtrix.org) (28) utilizing FSL v5.0.11. A longitudinal registration approach (29) was used to extract diffusion tensor metrics including FA, MD, RD, and AD from twenty white matter regions of interest (ROI) representing ten unique white matter tracts (30) (Table 2). White matter tracts were selected apriori according to previously reported associations in ASD (10,13,15,26,31–36). See supplementary methods for additional detail and summary of head motion protocols.

Statistical Analyses

Linear mixed effects modeling was performed using *R* version 3.6 (R Core Team, 2019). Ten groupings of biologically related ROIs were separately modeled for each DW-MRI feature (Table 2). Each model included diagnosis, sex, pre/post scanner update and ROI as categorical fixed effect factors, age in months, total cerebral volume, mean absolute and relative movement as continuous covariates, and individual as a random effect with age as a random slope. To determine the optimal form of age, a range of polynomials (−3,−2,−1,−0.5,0.5,1,2,3) sufficient to estimate most curvilinear trajectories were fit (37). The factored age term shown to have the lowest log likelihood was then selected to model age for that grouping of ROIs (Table 2).

In addition, all models included an age-by-diagnosis interaction term to test for differences between ASD and TD individuals in the developmental trajectory of DW-MRI measures, a diagnosis-by-ROI term to test for diagnostic differences between grouped ROIs, and an age-by-ROI term to account for potential developmental differences between grouped ROIs. Additionally, main effects of diagnosis were estimated at the mean age of time 1 (37.1 months) and time 3 (63.6 months). Multiple comparisons were corrected using the false discovery rate approach (FDR) (38). Given the primary aim to investigate FA differences, FDR was applied across each term within each DW-MRI feature (n=10). To investigate potential diagnostic sex differences, the significance of adding diagnosis-by-sex-by-age-by-ROI, diagnosis-by-sex-by-age, diagnosis-by-sex-by-ROI, and diagnosis-by-sex interactions to the above model were evaluated in a step-down fashion by maximum likelihood model comparisons using likelihood ratio tests.

Relationship between white matter development and autism severity

Secondary analyses were conducted to investigate associations between white matter development and both baseline calibrated autism severity scores (39) and changes in these scores over time. For the analysis of baseline severity, the fixed factor diagnosis term was substituted with a continuous baseline ADOS severity term. To investigate relationships between white matter development and changes in autism severity over time, a subset of participants with ASD who completed ADOS assessments at both time one and three (n=65♂,30♀) were categorized into one of three groups. Details on how these groups were formulated and associated behavioral differences between them (e.g. IQ) have been described previously (20). In brief, groups were divided according to 1) individuals who decreased in calibrated severity score by 2 or more points (n=17♂,10♀), 2) individuals whose scores changed by one point or less (n=34♂,16♀), and 3) individuals who increased in severity by 2 or more points (n=14♂,4♀) (Figure 1). Significant differences in white matter development between individuals with ASD who decreased, remained stable, or increased in symptom severity were evaluated by substituting a factorial term coding the three ADOS severity change groups for the diagnosis term in the mixed effects model and adding baseline ADOS severity at time of enrollment as a continuous factor.

Results

Demographics

ASD and TD participants did not significantly differ in the number of DW-MRI timepoints acquired, nor in age across all timepoints, at time one, time two, or time three (p>0.05). At time one, individuals with ASD had significantly lower Mullen full scale, verbal, and non-verbal DQ scores compared to TD controls (p<.0001). Males and females with ASD did not significantly differ in baseline full scale, verbal or non-verbal DQ scores (p>0.05) or baseline ADOS calibrated, social affect, or restricted repetitive behavior severity scores (p>0.05). Across all timepoints, individuals with ASD and TD had no significant differences in either mean relative (p=0.33) or absolute (p=0.90) head motion. See supplement for additional details.

White matter regions with significant differences in developmental trajectory in autism

Significant age-by-diagnosis interactions in FA (FDR p<0.05), indicating differences between individuals with ASD and TD controls in FA development, were observed within the cingulum bundle, superior longitudinal fasciculus, and internal capsule. These tracts showed ‘slower’ FA development in ASD relative to TD controls (Table 2, Figure 2). The corpus callosum showed a significant (FDR p<0.05) diagnosis-by-ROI effect indicating developmental differences between the genu, body, and splenium associated with autism. Within the corpus callosum, the splenium (uncorrected p=0.001, FDR p=0.01) and body (uncorrected p=0.03, FDR p=0.21) both differed from the genu and exemplified the observed slower FA development in ASD, while in the genu the developmental trajectories of FA for ASD and TD individuals appeared similar (Figure 3).

Across ROIs, the middle and inferior cerebellar peduncles, superior longitudinal fasciculus, internal capsule, and splenium of the corpus callosum were found to have an estimated

developmental trajectory that predicted higher FA values in young autistic children, that due to slower development intersected the estimated developmental trajectory of TD children. After FDR correction, no significant differences in FA were observed between ASD and TD individuals at the mean age of the time 1 assessment (37.1 months). However, due to the slower development of FA in ASD, at time 3 (63.6 months) significant decreases in FA in ASD emerged in the sagittal stratum, cingulum, uncinate fasciculus, and internal capsule (Table 2).

Additionally, the uncinate fasciculus showed significant (FDR $p<0.05$) age-by-diagnosis interactions in measures of AD characterized by initial increases and later decreases in ASD compared to TD controls (Figure S1). No significant age-by-diagnosis interactions were observed for measures of MD or RD. While age-by-diagnosis effects did not reach statistical significance in all tracts for all measures, a consistent developmental trend in measures of FA, MD, RD, and AD could be observed between TD and ASD across a majority of white matter tracts (Figure S1, Table S2). Likelihood model comparisons of the initial model and models with terms defining interactions with sex did not show significant (FDR $p>0.05$) differences.

Associations between white matter development and ADOS severity

Within the sagittal stratum, autistic individuals who increased in calibrated ADOS severity scores by two or more points from timepoint one to three had a significantly different (uncorrected $p=0.001$, FDR $p=0.01$) developmental trajectory in FA compared to the decreased severity group and those that remained stable (uncorrected $p=0.02$, FDR $p=0.27$), however, the later finding did not survive FDR $p<0.05$ correction. Individuals who increased in severity showed a developmental trajectory characterized by early increases in FA with slower development resulting in later FA decreases compared to the stable and decreased severity groups. Trend level differences between the severity change groups were also observed within the uncinate fasciculus where individuals who decreased in ADOS severity showed greater gains in FA compared to those who increased in severity (uncorrected $p=0.01$, FDR $p=0.06$) or remained stable (uncorrected $p=0.02$, FDR $p=0.20$) (Figure 4). Similar developmental trends between ADOS change groups were observed for FA across many of the tracts studied, but no further effects reached statistical significance (Figure S2, Table S3). No significant associations between FA, MD, RD or AD and baseline ADOS severity or baseline ADOS severity-by-age were observed.

Discussion

Our aim was to characterize the development of white matter structure in ASD compared to TD across early childhood. We found the developmental trajectory of FA measures from approximately 2.5-7 in ASD to be slower compared to TD, leading to the emergence of lower FA values in ASD with age. Across early childhood the developmental trajectory of FA in ASD was marked by a transition from increased to decreased FA compared to TD in five white matter tracts. Furthermore, we found significant differences in white matter development between individuals who increased, remained stable, or decreased in measures of autism severity across childhood. Within the sagittal stratum, autistic individuals who

increased in severity had a developmental trajectory that predicted early FA increases and slower FA development compared to those who decreased in symptom severity. Taken together these findings: 1) support atypical white matter structure as a neurophenotypic trait in ASD, 2) help establish the developmental course of white matter structure across childhood, and 3) highlight a functional relationship between white matter development and symptom course in ASD.

In the current study we found atypical development of FA measures within several large white matter tracts previously indicated in autism. The corpus callosum has been found to have atypical structure and diffusion properties in ASD (33,34) which have been associated with social deficits in the condition (35) and found to predictive of repetitive behaviors and sensory responsiveness in high risk infants (40). The superior longitudinal fasciculus, a large associative fiber tract that provides frontal-parietal connections, has also been indicated in ASD (50) and correlated with executive functioning, attention processing, and language ability (41). The internal capsule, which connects regions of the prefrontal cortex to the thalamus, basal ganglia, cerebellum and spinal cord, has been associated with both motor deficits (13) and core symptoms in ASD (36). Lastly, the cingulum bundle has been widely implicated in ASD and associated domains such as social cognition, emotion processing, and motor control (32). Additionally, we observed that slower FA development led to the emergence of significantly lower FA measures in ASD by the age of five years in tracts including the internal capsule and cingulum bundle. This suggests that previously reported FA decreases in older children, adolescents and adults with ASD develop across early childhood.

While significant increases in FA were not found at the mean age of time one (37 months), within the splenium of the corpus callosum, superior longitudinal fasciculus, internal capsule and middle and inferior cerebellar peduncles, we observed that the developmental trajectory of FA in ASD was marked by early increases that, due to a slower developmental course, intersected the TD trajectory resulting in later comparative decreases in FA. While not all tracts in the current study indicated a developmental transition from increased to decreased FA in ASD compared to TD, the inflection points observed for tracts that did were between 30-50 months of age, in line with the only previous longitudinal DW-MRI report to focus on this period of development (8). Studies of high-risk infant siblings suggest increased FA in ASD may transition to decreases prior to 24 months in some cases (6) meaning that this effect may have occurred in certain tracts prior to the age covered by this study.

While ASD is believed to first biologically manifest prenatally (42,43) it is increasingly understood that ASD symptoms continue to emerge and change across childhood (44). Prolonged manifestation of the autism phenotype, environmental variability, and individual differences in levels of intervention and intervention susceptibility potentially explain clinically relevant changes in ASD severity over time (19,20). Here we found that compared to other autistic children, individuals who increased in autism symptom severity experienced a concurrent slower development of FA measures in white matter. This developmental relationship was observed across most white matter tracts studied, however after FDR correction only reached statistical significance within the sagittal stratum. The sagittal stratum is a fiber complex that incorporates the inferior longitudinal fasciculus, inferior

fronto-occipital fasciculus and posterior thalamic radiation, and connects parietal, occipital, and temporal cortical regions to thalamic and other subcortical structures. This fiber complex has been previously indicated in ASD (45) and functionally linked to visually guided behavior such as non-verbal semantic processing (46) and face-based mentalizing (47). Additionally, within the uncinate fasciculus a trend was observed in which individuals who decreased in autism severity had greater FA gains. The uncinate fasciculus is a medial frontotemporal connection also previously implicated in ASD (31) and in aspects of social interaction, e.g. joint attention (48).

Few other studies have directly investigated relationships between longitudinal changes in white matter structure and autism severity change. One recent study reported that higher frontoparietal network structural connectivity during late childhood/adolescence was predictive of lower symptom load in adolescence and early adulthood (49). Despite covering a later age range, this study is comparable to the current findings in that a relationship between symptom change and white matter structure was observed regardless of baseline severity measures. However, others have reported a positive association between FA and ADOS social communication scores from 12-28 months but a negative association at 37-48 months (8). Collectively these findings suggest a functional relationship between white matter development and core autism symptoms whereby the current results indicate that slower FA development may represent a potential marker of a worsening trajectory of autism symptoms. No significant differences in intervention hours or intensity were observed within the APP/GAIN cohort between the increased, stable, and decreased severity groups (20). However, further study is needed to determine if the underlying processes resulting in atypical white matter structure are a causal factor in ASD symptom severity increases or if altered white matter development in the condition results from other disrupted processes, such as atypical local/distal connectivity efficiency (50), or shared environmental factors.

The maturation of white matter is a dynamic process that involves multiple neurobiological mechanisms including axonal proliferation, pruning, and myelination (51) that may impact DW-MRI measures (14). One potential explanatory factor for previously reported early life increases in FA in ASD is excessive axonal projections and density. Several lines of research indicate accelerated cortical growth in ASD (50,52,53) which would be predicted to result in increased axonal projections (54). Recently, a large study of TD young adults reported FA in the corpus callosum to be more strongly associated with proxy measures of axonal density compared to myelination, although heterogeneity in the spatial distribution of these associations across callosal regions was noted (55). Histological studies of white matter in ASD needed to confirm underlying structural deficits in ASD are limited but do report increased numbers of thin axonal fibers and reduced myelination (56,57). While a majority of myelination occurs prior to the age of five, this process follows a protracted developmental course that extends into adulthood (51). Thus, in ASD one could hypothesize that processes contributing to axonal overgrowth early in life also result in signal delays and metabolic inefficiencies in connecting disparate brain regions (50). These atypical processes could result in less integrated and distributed neural networks, which have been reported in high risk infants with ASD (58), leading to slower development and/or loss of myelin over time.

To our knowledge, this study represents the largest longitudinal sample of young children with ASD to participate in a DW-MRI study. Inclusion of individuals with more significant autism related impairments and a relatively large number of autistic females makes the sample more representative of the broader autism population than most imaging studies. However, the number of female participants limited the ability to investigate sex differences between autism symptom change groups. With larger samples this would be an important future direction for additional study. It should also be noted that we report longitudinal findings over a highly dynamic period of early childhood development. However, white matter development is widely accepted to continue throughout adolescents and into early adulthood. Currently observed group differences in white matter development were largely found within large white matter tracts that are believed to undergo most of their development early in life (51). Further longitudinal studies will be needed to identify differences that may emerge at later ages (e.g. in frontal tracts, peripheral white matter). Such studies would be beneficial for understanding the full developmental course of white matter in ASD and its potential relationships with adolescent and adult outcomes.

In the current study we employed an atlas-based approach which limits the definition of certain ROIs to areas of the associated white matter tract that can be discretely dissected. For example, the uncinate fasciculus ROI includes only the fibers linking the frontal and temporal lobes and not the tract's further projections into frontal and temporal regions where it merges and becomes indistinguishable (using tensor modeling) from other tracts. Given the limitations of tensor based tractography, this approach increases confidence that measures are being taken from the tract of interest and not a conglomerate of interdigitated tracts. However, future studies should consider advanced acquisition sequences such as High Angular Resolution Diffusion Imaging (HARDI) (59) and techniques (60) that more accurately model diffusion within complex fiber orientations. Such studies should also consider utilizing multimodal imaging sequences to concurrently investigate different aspects of white matter structure in order to better address the non-specific relationship of DW-MRI measures with underlying biology (51,55). These future studies would also benefit by investigating multimodal interrelationships between white and grey matter development to provide a more holistic view of brain development in ASD. We should also note that FDR was selected apriori to control for multiple comparisons over a more conservative Bonferroni approach as this study sought to concurrently investigate development across several white matter tracts. However, many of the currently reported findings would have met a similarly applied Bonferroni correction ($p<0.005$, for 10 comparisons).

We report the largest longitudinal DW-MRI study to date of white matter development in autistic participants across early childhood. Findings of a slower developmental trajectory of FA measures in ASD resulting in emerging decreases in FA with age, suggest that previously reported differences between cross-sectional studies of younger and older autism cohorts are attributable to alterations in dynamic biological processes of white matter development and are not due to sampling differences. Furthermore, significant differences in white matter development between individuals who increased, remained stable, or decreased in autism severity indicate that the structural integrity of connections within the brain are intrinsically related to phenotypic outcomes in autism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References:

1. Association AP. Diagnostic and Statistical Manual of Mental Disorders (DSM-5®). American Psychiatric Pub; 2013 1520 p.
2. Picci G, Gotts SJ, Scherf KS. A theoretical rut: revisiting and critically evaluating the generalized under/over-connectivity hypothesis of autism. Dev Sci. 2016;19(4):524–49. [PubMed: 27412228]
3. Ben Bashat D, Kronfeld-Duenias V, Zachor DA, Ekstein PM, Hendler T, Tarrasch R, et al. Accelerated maturation of white matter in young children with autism: A high b value DWI study. Neuroimage. 2007 8 1;37(1):40–7. [PubMed: 17566764]
4. Weinstein M, Ben-Sira L, Levy Y, Zachor DA, Itzhak EB, Artzi M, et al. Abnormal white matter integrity in young children with autism. Hum Brain Mapp. 2011;32(4):534–43. [PubMed: 21391246]
5. Billeci L, Calderoni S, Tosetti M, Catani M, Muratori F. White matter connectivity in children with autism spectrum disorders: a tract-based spatial statistics study. BMC Neurol. 2012 11 29;12(1):148. [PubMed: 23194030]
6. Wolff JJ, Gu H, Gerig G, Elison JT, Styner M, Gouttard S, et al. Differences in White Matter Fiber Tract Development Present From 6 to 24 Months in Infants With Autism. Am J Psychiatry. 2012 6 1;169(6):589–600. [PubMed: 22362397]
7. Xiao Z, Qiu T, Ke X, Xiao X, Xiao T, Liang F, et al. Autism Spectrum Disorder as Early Neurodevelopmental Disorder: Evidence from the Brain Imaging Abnormalities in 2–3 Years Old Toddlers. J Autism Dev Disord. 2014 7 1;44(7):1633–40. [PubMed: 24419870]
8. Solso S, Xu R, Proudfoot J, Hagler DJ, Campbell K, Venkatraman V, et al. Diffusion Tensor Imaging Provides Evidence of Possible Axonal Overconnectivity in Frontal Lobes in Autism Spectrum Disorder Toddlers. Biol Psychiatry. 2016 4 15;79(8):676–84. [PubMed: 26300272]
9. Fingher N, Dinstein I, Ben-Shachar M, Haar S, Dale AM, Eyler L, et al. Toddlers later diagnosed with autism exhibit multiple structural abnormalities in temporal corpus callosum fibers. Cortex. 2017 12 1;97:291–305. [PubMed: 28202133]
10. Andrews DS, Lee JK, Solomon M, Rogers SJ, Amaral DG, Nordahl CW. A diffusion-weighted imaging tract-based spatial statistics study of autism spectrum disorder in preschool-aged children. J Neurodev Disord. 2019 12 16;11(1):32. [PubMed: 31839001]
11. Conti E, Calderoni S, Marchi V, Muratori F, Cioni G, Guzzetta A. The first 1000 days of the autistic brain: a systematic review of diffusion imaging studies. Front Hum Neurosci [Internet]. 2015 [cited 2020 May 26];9 Available from: https://www.frontiersin.org/articles/10.3389/fnhum.2015.00159/full
12. Travers BG, Tromp DPM, Adluru N, Lange N, Destiche D, Ennis C, et al. Atypical development of white matter microstructure of the corpus callosum in males with autism: a longitudinal investigation. Mol Autism. 2015 3 11;6(1):15. [PubMed: 25774283]
13. McLaughlin K, Travers BG, Dadalko OI, Dean DC, Tromp D, Adluru N, et al. Longitudinal development of thalamic and internal capsule microstructure in autism spectrum disorder. Autism Res. 2018;11(3):450–62. [PubMed: 29251836]
14. Jones DK, Knösche TR, Turner R. White matter integrity, fiber count, and other fallacies: The do's and don'ts of diffusion MRI. NeuroImage. 2013 6 1;73:239–54. [PubMed: 22846632]

15. Ameis SH, Catani M. Altered white matter connectivity as a neural substrate for social impairment in Autism Spectrum Disorder. Cortex. 2015 1 1 ;62:158–81. [PubMed: 25433958]
16. Hong S-J, Hyung B, Paquola C, Bernhardt BC. The Superficial White Matter in Autism and Its Role in Connectivity Anomalies and Symptom Severity. Cereb Cortex. 2019 9 13;29(10):4415–25. [PubMed: 30566613]
17. Cheng Y, Chou K-H, Chen I-Y, Fan Y-T, Decety J, Lin C-P. Atypical development of white matter microstructure in adolescents with autism spectrum disorders. NeuroImage. 2010 4 15;50(3):873–82. [PubMed: 20074650]
18. Fitzgerald J, Leemans A, Kehoe E, O'Hanlon E, Gallagher L, McGrath J. Abnormal frontoparietal white matter organisation in the superior longitudinal fasciculus branches in autism spectrum disorders. Eur J Neurosci. 2018;47(6):652–61. [PubMed: 28741714]
19. Gotham K, Pickles A, Lord C. Trajectories of Autism Severity in Children Using Standardized ADOS Scores. Pediatrics. 2012 11 1;130(5):e1278–84. [PubMed: 23090336]
20. Waizbard-Bartov E, Ferrer E, Young GS, Heath B, Rogers S, Wu Nordahl C, et al. Trajectories of Autism Symptom Severity Change During Early Childhood. J Autism Dev Disord [Internet]. 2020 5 14 [cited 2020 May 20]; Available from: http://link.springer.com/10.1007/s10803-020-04526-z
21. Lord C, Risi S, Lambrecht L, Cook EH, Leventhal BL, DiLavore PC, et al. The Autism Diagnostic Observation Schedule—Generic: A Standard Measure of Social and Communication Deficits Associated with the Spectrum of Autism. J Autism Dev Disord. 2000 6 1;30(3):205–23. [PubMed: 11055457]
22. Lord C, Rutter M, DiLavore P, Risi S, Gotham K, Bishop S. Autism diagnostic observation schedule-2nd edition (ADOS-2). Los Angel CA West Psychol Corp 2012;
23. Lord C, Rutter M, Le Couteur A. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. J Autism Dev Disord. 1994;24(5):659–85. [PubMed: 7814313]
24. Mullen EM. Mullen scales of early learning. AGS Circle Pines, MN; 1995.
25. Johnson RT, Yeatman JD, Wandell BA, Buonocore MH, Amaral DG, Nordahl CW. Diffusion properties of major white matter tracts in young, typically developing children. NeuroImage. 2014 3 1;88:143–54. [PubMed: 24269274]
26. Nordahl CW, Iosif A-M, Young GS, Perry LM, Dougherty R, Lee A, et al. Sex differences in the corpus callosum in preschool-aged children with autism spectrum disorder. Mol Autism. 2015 5 13;6(1):26. [PubMed: 25973163]
27. Nordahl CW, Simon TJ, Zierhut C, Solomon M, Rogers SJ, Amaral DG. Brief Report: Methods for Acquiring Structural MRI Data in Very Young Children with Autism Without the Use of Sedation. J Autism Dev Disord. 2008 9 1;38(8):1581–90. [PubMed: 18157624]
28. Tournier J-D, Smith R, Raffelt D, Tabbara R, Dhollander T, Pietsch M, et al. MRtrix3: A fast, flexible and open software framework for medical image processing and visualisation. Neuroimage. 2019 11 15;202:116137. [PubMed: 31473352]
29. Aubert-Broche B, Fonov VS, García-Lorenzo D, Mouiha A, Guizard N, Coupé P, et al. A new method for structural volume analysis of longitudinal brain MRI data and its application in studying the growth trajectories of anatomical brain structures in childhood. NeuroImage. 2013 11 15;82:393–402. [PubMed: 23719155]
30. Mori S, Oishi K, Jiang H, Jiang L, Li X, Akhter K, et al. Stereotaxic white matter atlas based on diffusion tensor imaging in an ICBM template. NeuroImage. 2008 4 1;40(2):570–82. [PubMed: 18255316]
31. Catani M, Dell'Acqua F, Budisavljevic S, Howells H, Thiebaut de Schotten M, Froudist-Walsh S, et al. Frontal networks in adults with autism spectrum disorder. Brain. 2016 2 1;139(2):616–30. [PubMed: 26912520]
32. Hau J, Aljawad S, Baggett N, Fishman I, Carper RA, Müller R-A. The cingulum and cingulate U-fibers in children and adolescents with autism spectrum disorders. Hum Brain Mapp. 2019;40(11):3153–64. [PubMed: 30941791]
33. Alexander AL, Lee JE, Lazar M, Boudos R, DuBray MB, Oakes TR, et al. Diffusion tensor imaging of the corpus callosum in Autism. NeuroImage. 2007 1 1;34(1):61–73. [PubMed: 17023185]

34. Aoki Y, Abe O, Nippashi Y, Yamasue H. Comparison of white matter integrity between autism spectrum disorder subjects and typically developing individuals: a meta-analysis of diffusion tensor imaging tractography studies. Mol Autism. 2013 7 22;4(1):25. [PubMed: 23876131]
35. Aoki Y, Yoncheva YN, Chen B, Nath T, Sharp D, Lazar M, et al. Association of White Matter Structure With Autism Spectrum Disorder and Attention-Deficit/Hyperactivity Disorder. JAMA Psychiatry. 2017 11 1;74(11):1120–8. [PubMed: 28877317]
36. Cheon K-A, Kim Y-S, Oh S-H, Park S-Y, Yoon H-W, Herrington J, et al. Involvement of the anterior thalamic radiation in boys with high functioning autism spectrum disorders: A Diffusion Tensor Imaging study. Brain Res. 2011 10 12;1417:77–86. [PubMed: 21890117]
37. Long J, Ryoo J. Using fractional polynomials to model non-linear trends in longitudinal data. Br J Math Stat Psychol. 2010;63(1):177–203. [PubMed: 19486548]
38. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J R Stat Soc Ser B Methodol. 1995;57(1):289–300.
39. Gotham K, Pickles A, Lord C. Standardizing ADOS Scores for a Measure of Severity in Autism Spectrum Disorders. J Autism Dev Disord. 2009 5 1;39(5):693–705. [PubMed: 19082876]
40. Wolff JJ, Swanson MR, Elison JT, Gerig G, Pruett JR, Styner MA, et al. Neural circuitry at age 6 months associated with later repetitive behavior and sensory responsiveness in autism. Mol Autism. 2017 3 4;8(1):8. [PubMed: 28316772]
41. Urger SE, De Bellis MD, Hooper SR, Woolley DP, Chen SD, Provenzale J. The Superior Longitudinal Fasciculus in Typically Developing Children and Adolescents: Diffusion Tensor Imaging and Neuropsychological Correlates. J Child Neurol. 2015 1 1;30(1):9–20. [PubMed: 24556549]
42. An J-Y, Lin K, Zhu L, Werling DM, Dong S, Brand H, et al. Genome-wide de novo risk score implicates promoter variation in autism spectrum disorder. Science [Internet]. 2018 12 14 [cited 2020 Apr 14];362(6420). Available from: https://science.sciencemag.org/content/362/6420/eaat6576
43. Satterstrom FK, Kosmicki JA, Wang J, Breen MS, De Rubeis S, An J-Y, et al. Large-Scale Exome Sequencing Study Implicates Both Developmental and Functional Changes in the Neurobiology of Autism. Cell. 2020 2 6;180(3):568–584.e23. [PubMed: 31981491]
44. Ozonoff S, Gangi D, Hanzel EP, Hill A, Hill MM, Miller M, et al. Onset patterns in autism: Variation across informants, methods, and timing. Autism Res. 2018;11(5):788–97. [PubMed: 29524310]
45. Hattori A, Kamagata K, Kirino E, Andica C, Tanaka S, Hagiwara A, et al. White matter alterations in adult with autism spectrum disorder evaluated using diffusion kurtosis imaging. Neuroradiology. 2019 12 1;61(12):1343–53. [PubMed: 31209529]
46. Herbet G, Moritz-Gasser S, Duffau H. Direct evidence for the contributive role of the right inferior fronto-occipital fasciculus in non-verbal semantic cognition. Brain Struct Funct. 2017 5 1;222(4):1597–610. [PubMed: 27568379]
47. Yordanova YN, Duffau H, Herbet G. Neural pathways subserving face-based mentalizing. Brain Struct Funct. 2017 9 1;222(7):3087–105. [PubMed: 28243761]
48. Elison JT, Wolff JJ, Heimer DC, Paterson SJ, Gu H, Hazlett HC, et al. Frontolimbic neural circuitry at 6 months predicts individual differences in joint attention at 9 months. Dev Sci. 2013;16(2) :186–97. [PubMed: 23432829]
49. Lin H-Y, Perry A, Cocchi L, Roberts JA, Tseng W-YI, Breakspear M, et al. Development of frontoparietal connectivity predicts longitudinal symptom changes in young people with autism spectrum disorder. Transl Psychiatry. 2019 2 12;9(1):1–10. [PubMed: 30664621]
50. Casanova MF, Buxhoeveden D, Gomez J. Disruption in the Inhibitory Architecture of the Cell Minicolumn: Implications for Autisim. The Neuroscientist. 2003 12 1;9(6):496–507. [PubMed: 14678582]
51. Lebel C, Deoni S. The development of brain white matter microstructure. NeuroImage. 2018 11 15;182:207–18. [PubMed: 29305910]
52. Hazlett HC, Gu H, Munsell BC, Kim SH, Styner M, Wolff JJ, et al. Early brain development in infants at high risk for autism spectrum disorder. Nature. 2017 2;542(7641):348–51. [PubMed: 28202961]

53. Wang M, Wei P-C, Lim CK, Gallina IS, Marshall S, Marchetto MC, et al. Increased Neural Progenitor Proliferation in a hiPSC Model of Autism Induces Replication Stress-Associated Genome Instability. Cell Stem Cell. 2020 2 6;26(2):221–233.e6. [PubMed: 32004479]

54. Ringo JL. Neuronal Interconnection as a Function of Brain Size. Brain Behav Evol. 1991 ;38(1):1–6. [PubMed: 1657274]

55. Friedrich P, Fraenz C, Schlüter C, Ocklenburg S, Mädler B, Güntürkün O, et al. The Relationship Between Axon Density, Myelination, and Fractional Anisotropy in the Human Corpus Callosum. Cereb Cortex [Internet]. [cited 2020 Apr 14]; Available from: https://academic.oup.com/cercor/advance-article/doi/10.1093/cercor/bhz221/5652191

56. Zikopoulos B, Barbas H. Changes in Prefrontal Axons May Disrupt the Network in Autism. J Neurosci. 2010 11 3;30(44):14595–609. [PubMed: 21048117]

57. Wegiel J, Kaczmarski W, Flory M, Martinez-Cerdeno V, Wisniewski T, Nowicki K, et al. Deficit of corpus callosum axons, reduced axon diameter and decreased area are markers of abnormal development of interhemispheric connections in autistic subjects. Acta Neuropathol Commun. 2018 12 19;6(1):143. [PubMed: 30567587]

58. Lewis JD, Evans AC, Pruett JR, Botteron KN, McKinstry RC, Zwaigenbaum L, et al. The Emergence of Network Inefficiencies in Infants With Autism Spectrum Disorder. Biol Psychiatry. 2017 01;82(3):176–85. [PubMed: 28460842]

59. Descoteaux M, Angelino E, Fitzgibbons S, Deriche R. Apparent diffusion coefficients from high angular resolution diffusion imaging: Estimation and applications. Magn Reson Med. 2006;56(2):395–410. [PubMed: 16802316]

60. Raffelt DA, Tournier J-D, Smith RE, Vaughan DN, Jackson G, Ridgway GR, et al. Investigating white matter fibre density and morphology using fixel-based analysis. NeuroImage. 2017 1 1;144:58–73. [PubMed: 27639350]

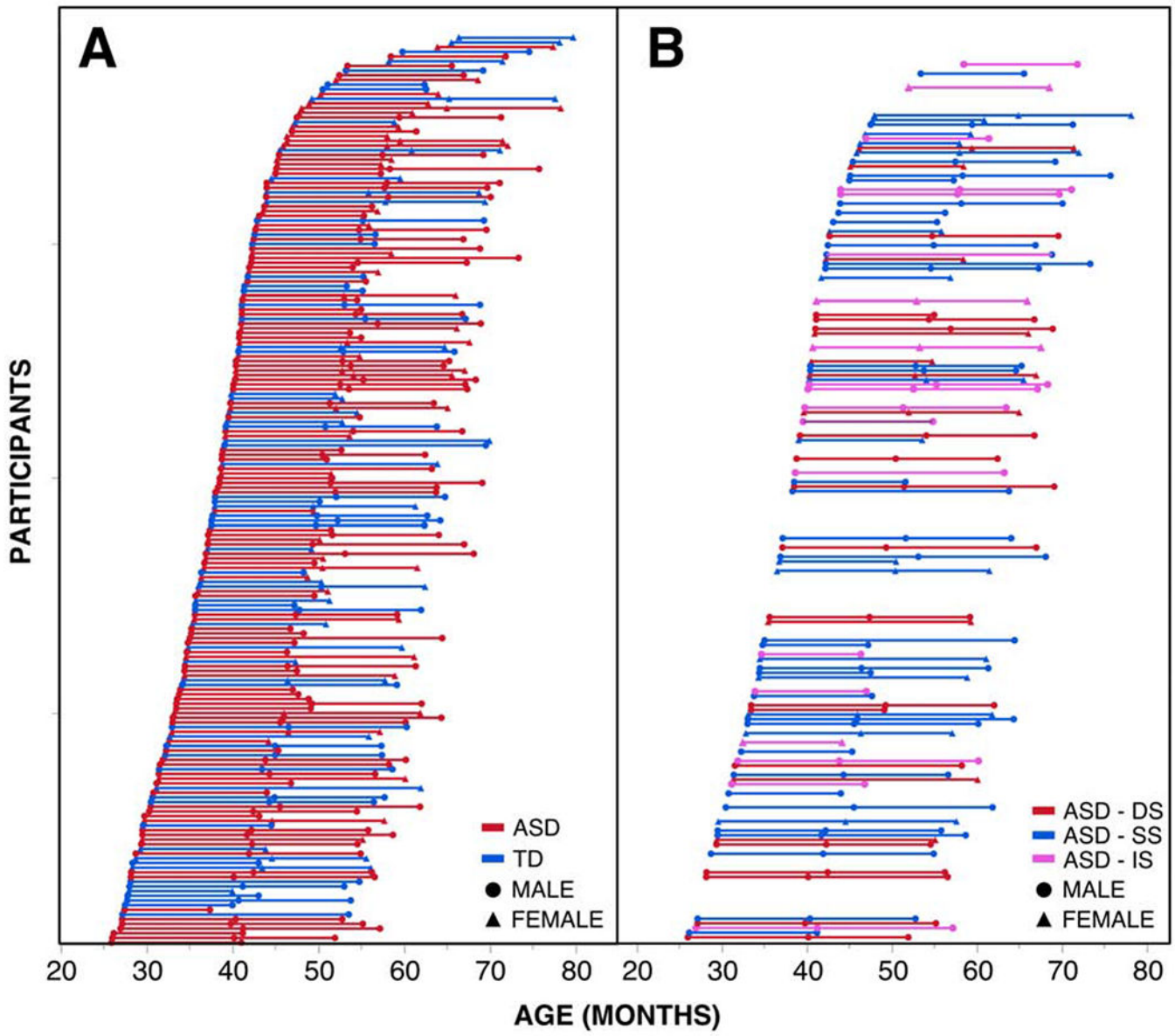

Figure 1: Longitudinal Cohort

A) Individual's timepoints are plotted according to diagnosis and sex. To investigate relationships between white matter development and changes in autism severity B) a subsample of autistic participants who completed ADOS assessment at study time 1 and 3 were defined. This sample consisted of three groups 1) decreased severity (DS), 2) stable severity (SS), and 3) increased severity (IS). ASD=autism spectrum disorder, TD='typical development'.

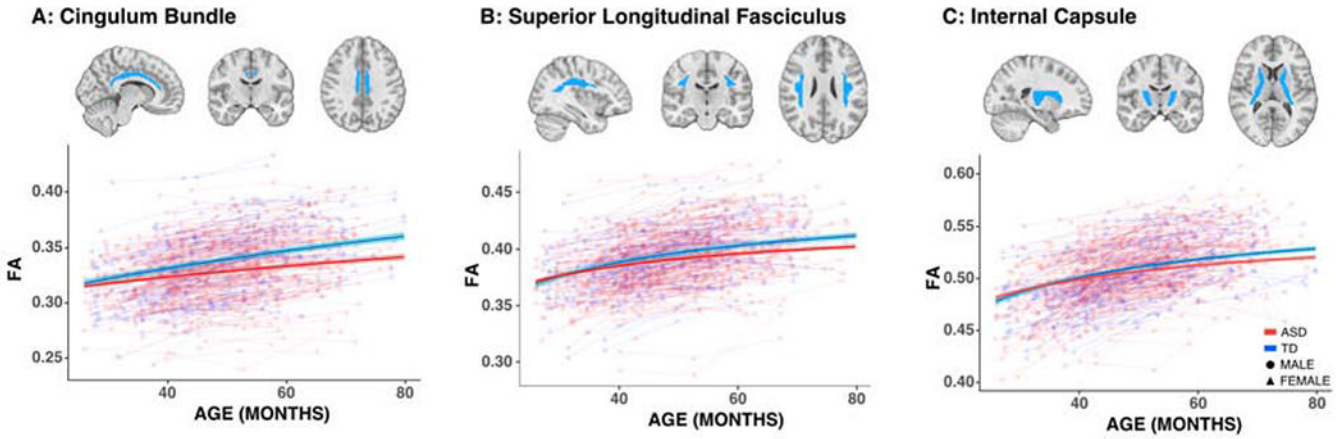

Figure 2: Diagnostic Group by Age Interactions in Measures of Fractional Anisotropy

A) The cingulum bundle, B) superior longitudinal fasciculus, and C) internal capsule showed significant (FDR $p<0.05$) interaction effects indicating differences in the developmental trajectory of fractional anisotropy (FA) between typically development (TD) and individuals with autism spectrum disorder (ASD).

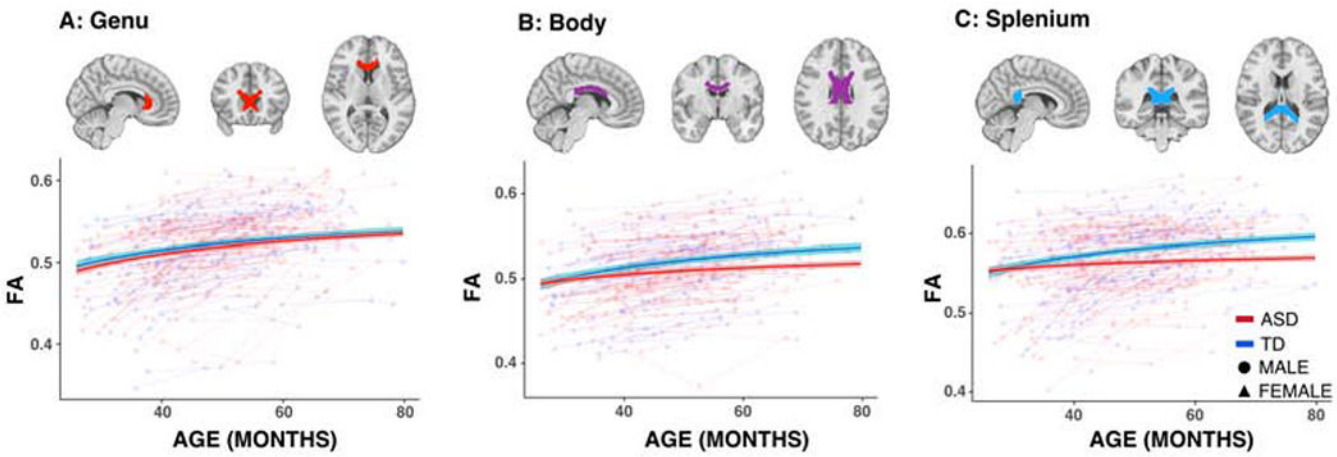

Figure 3: Fractional Anisotropy Development in the Corpus Callosum

Significant diagnosis-by-ROI effects indicated diagnostic differences between the A) genu, B) body, and C) splenium of the corpus callosum. Within the corpus callosum the splenium (uncorrected p=0.001, FDR p=0.01) and body (uncorrected p=0.03, FDR p=0.21) both differed from the genu and exemplified the observed slower development of FA in ASD while the developmental trajectories of FA for ASD and TD individuals in the genu appear similar.

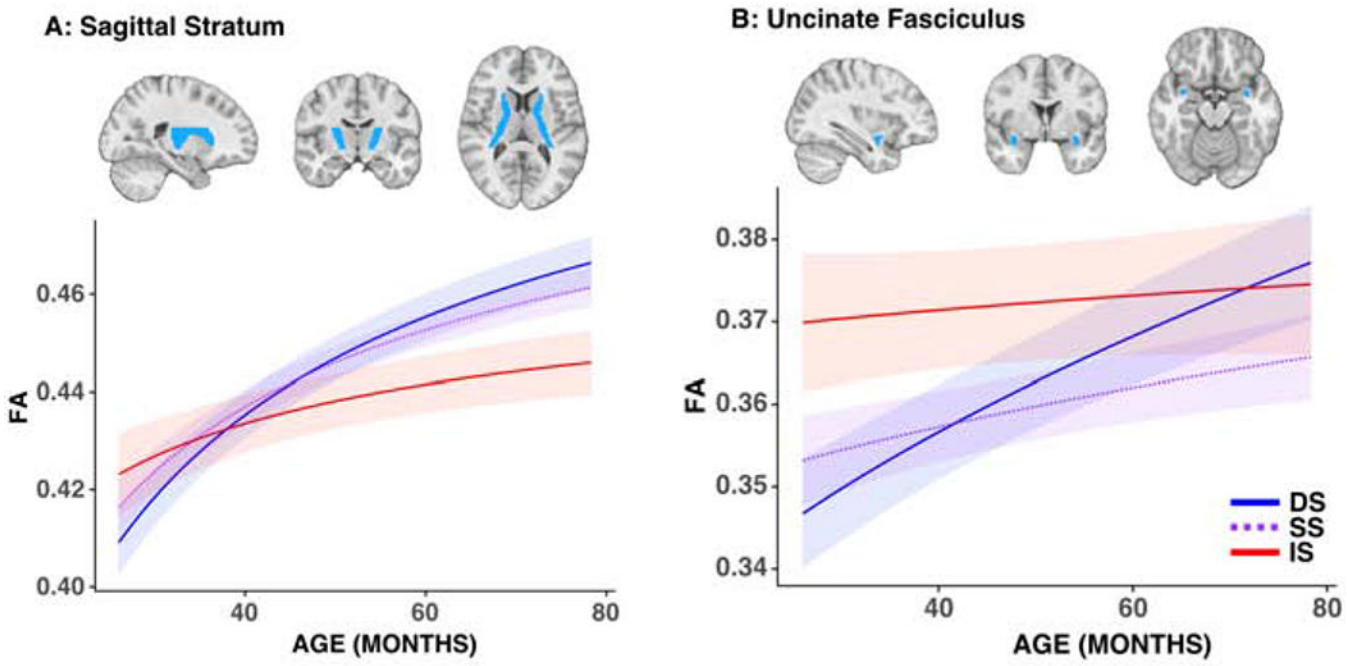

Figure 4: Relationship of Fractional Anisotropy Development and Changes in Autism Severity

A) Within the sagittal stratum, autistic individuals who increased in calibrated ADOS severity scores (IS) significantly differed in the developmental trajectory of fractional anisotropy (FA) measures compared to those who decreased in severity (DS) (uncorrected p=0.001, FDR p=0.01). B) Within the uncinate fasciculus, a trend was observed in which the IS group showed less gains in FA and significantly differed from the DS group (uncorrected p=0.01). Furthermore, in the uncinate fasciculus the DS group showed greater gains in FA and significantly differed from the SS group (uncorrected p=0.02).

Table 1:

Participant Demographics

	Full Sample		Males		Females	
	ASD (n=125)	**TD (n=69)**	**ASD (n=85)**	**TD (n=38)**	**ASD (n=40)**	**TD (n=31)**
DQ	66.03 (22.31)	107.07 (11.92)	65.10 (21.32)	103.69 (12.52)	68.06 (24.16)	111.22 (9.83)
ADOS Calibrated Severity	7.26 (1.79)	-	7.32 (1.75)	-	7.13 (1.86)	-
Absolute Movement (mm)	0.46 (0.54)	0.47 (0.38)	0.44 (0.42)	0.46 (0.32)	0.51 (0.74)	0.48 (0.45)
Relative Movement (mm)	0.36 (0.10)	0.37 (0.12)	0.36 (0.10)	0.37 (0.10)	0.36 (0.12)	0.36 (0.15)
Scans per participant	2.44 (0.50)	2.41 (0.49)	2.51 (0.50)	2.47 (0.51)	2.30 (0.46)	2.32 (0.48)
Scans at Time 1	119	60	81	33	38	27
Scans at Time 2	113	60	79	34	34	26
Scans at Time 3	73	46	53	27	20	19
Total number of Scans	305	166	213	94	92	72
Number of participants with three scans	70	41	43	18	12	10
Number of participants with two scans	55	28	42	20	28	21

Note: Values are given as mean (standard deviation). ASD=Autism Spectrum Disorder, TD=Typically Development, DQ=Mullen Developmental Quotient, ADOS=Autism Diagnostic Observation Schedule. DQ and ADOS scores reflect average at baseline time one assessment.

Table 2:

White Matter Regions of Interest and Diagnosis Effects

		FA, Age - by - Diagnosis Effects						FA, Time 1 Diagnosis Effects					FA, Time 3 Diagnosis Effects				
ROI Group	**White Matter ROI**	**Age Term**	**Coeff.**	**Std. Error**	**DF**	***t***	***p***	**Coeff.**	**Std. Error**	**DF**	***t***	***p***	**Coeff.**	**Std. Error**	**DF**	***t***	***p***
1	Middle Cerebellar Peduncle	0.5	−0.1280	0.066	271	−1.92	0.055	−0.0017	0.002	191	−0.65	0.516	0.0032	0.003	191	1.06	0.287
2	Inferior cerebellar peduncle R Inferior cerebellar peduncle L	0.5	0.0031	0.001	739	1.70	0.089	0.0024	0.003	191	0.66	0.504	0.0084	0.003	191	2.15	0.032*
3	Superior cerebellar peduncle R Superior cerebellar peduncle L	0.5	0.0007	0.001	739	0.36	0.719	0.0030	0.004	191	0.71	0.477	0.0043	0.004	191	0.94	0.345
4	Genu of Corpus Callosum Body of Corpus Callosum Splenium of Corpus Callosum	−0.5	−0.1780	0.080	1207	−2.2	0.027*	0.0012	0.005	191	0.24	0.808	0.0081	0.005	191	1.51	0.13
5	Sagittal stratum R Sagittal stratum L	−0.5	−0.0823	0.066	739	−1.25	0.213	0.0072	0.003	191	1.86	0.064	0.0104	0.003	191	2.63	0.009*
6	Cingulum R Cingulum L	0.5	0.0042	0.001	739	3.51	0.0004**	0.0072	0.003	191	2.07	0.039*	0.0151	0.003	191	3.97	<0.001*
7	Superior longitudinal fasciculus R Superior longitudinal fasciculus L	−0.5	−0.1420	0.049	739	−2.88	0.004**	0.0013	0.003	191	0.43	0.664	0.0068	0.003	191	2.09	0.037*
8	Superior fronto-occipital fasciculus R Superior fronto-occipital fasciculus L	0.5	0.0002	0.001	739	0.13	0.893	0.0028	0.004	191	0.65	0.513	0.0032	0.004	191	0.71	0.475
9	Uncinate fasciculus	0.5	0.0026	0.001	739	1.80	0.071	0.0086	0.004	191	1.86	0.063	0.0137	0.004	191	2.90	0.004*

			FA, Age - by - Diagnosis Effects					FA, Time 1 Diagnosis Effects					FA, Time 3 Diagnosis Effects				
ROI Group	**White Matter ROI**	**Age Term**	**Coeff.**	**Std. Error**	**DF**	***t***	***p***	**Coeff.**	**Std. Error**	**DF**	***t***	***p***	**Coeff.**	**Std. Error**	**DF**	***t***	***p***
	R Uncinate fasciculus L																
10	Internal Capsule R Internal Capsule L	−0.5	−0.1390	0.056	739	−2.47	0.0138 **	0.0025	0.002	191	0.88	0.375	0.0079	0.002	191	2.78	0.005 *

Note: Region of interests (ROI) were separately modelled according to ten anatomically related groups (ROI Group). R=Right, L=Left. Age term denotes the fractional polynomial term found to provide the best fit for the ROI group. Age-by-diagnosis, and diagnosis effects at the mean age of time 1 and time 3 assessments are given for the linear mixed effects model of fractional anisotropy (FA). DF=degrees of freedom.

* uncorrected $p<0.05$

** false discovery rate corrected $p<0.05$.

KEY RESOURCES TABLE

Resource Type	Specific Reagent or Resource	Source or Reference	Identifiers	Additional Information
Add additional rows as needed for each resource type	Include species and sex when applicable.	Include name of manufacturer, company, repository, individual, or research lab. Include PMID or DOI for references; use "this paper" if new.	Include catalog numbers, stock numbers, database IDs or accession numbers, and/or RRIDs. RRIDs are highly encouraged; search for RRIDs at https://scicrunch.org/resources.	Include any additional information or notes if necessary.
Deposited Data; Public Database	National Database for Autism Research	https://nda.nih.gov/about.html		https://nda.nih.gov/about.html
Software; Algorithm	MRtrix3	www.mrtrix.org/download		www.mrtrix.org
Software; Algorithm	FSL FDT	www.fsl.fmrib.ox.ac.uk		https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FDT
Software; Algorithm	NIVIDA CUDA	https://developer.nvidia.com/cuda-zone		https://developer.nvidia.com/cuda-zone
Software; Algorithm	Advanced Normalization Tools	https://stnava.github.io/ANTs/		https://stnava.github.io/ANTs/
Software; Algorithm	R	https://www.r-project.org/		https://www.r-project.org/
Software; Algorithm	R: nlme package	DouglasBatesbates@stat.wisc.edu		https://cran.r-project.org/web/packages/nlme/index.html
Other (Hardware)	3T Siemens Total Imaging Matrix Trio MR System	General Electric, Erlangen Germany		https://www.siemens-healthineers.com/
Other (Assessment)	Mullen Scales of Early Learning	WPS Publishing		https://www.wpspublish.com/mullen-scales-of-early-learning
Other (Assessment)	Autism Diagnostic Observation Schedule	WPS Publishing		https://www.wpspublish.com/ados-2-autism-diagnostic-observation-schedule-second-edition