Development of White Matter Circuitry in Infants
With Fragile X Syndrome

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IMPORTANCE Fragile X syndrome (FXS) is a genetic neurodevelopmental disorder and the most common inherited cause of intellectual disability in males. However, there are no published data on brain development in children with FXS during infancy.

OBJECTIVE To characterize the development of white matter at ages 6, 12, and 24 months in infants with FXS compared with that of typically developing controls.

DESIGN, SETTING, AND PARTICIPANTS Longitudinal behavioral and brain imaging data were collected at 1 or more time points from 27 infants with FXS and 73 typically developing controls between August 1, 2008, and June 14, 2016, at 2 academic medical centers. Infants in the control group had no first- or second-degree relatives with intellectual or psychiatric disorders, including FXS and autism spectrum disorder.

MAIN OUTCOMES AND MEASURES Nineteen major white matter pathways were defined in common atlas space based on anatomically informed methods. Diffusion parameters, including fractional anisotropy, were compared between groups using linear mixed effects modeling. Fiber pathways showing group differences were subsequently examined in association with direct measures of verbal and nonverbal development.

RESULTS There were significant differences in the development of 12 of 19 fiber tracts between the 27 infants with FXS (22 boys and 5 girls) and the 73 infants in the control group (46 boys and 27 girls), with lower fractional anisotropy in bilateral subcortical-frontal, occipital-temporal, temporal-frontal, and cerebellar-thalamic pathways, as well as 4 of 6 subdivisions of the corpus callosum. For all 12 of these pathways, there were significant main effects between groups but not for the interaction of age × group, indicating that lower fractional anisotropy was present and stable from age 6 months in infants with FXS. Lower fractional anisotropy values in the uncinate fasciculi were correlated with lower nonverbal developmental quotient in the FXS group (left uncinate, F = 10.06; false discovery rate–corrected P = .03; right uncinate, F = 21.8; P = .004).

CONCLUSIONS AND RELEVANCE The results substantiate in human infants the essential role of fragile X gene expression in the early development of white matter. The findings also suggest that the neurodevelopmental effects of FXS are well established at 6 months of age.

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F ragile X syndrome (FXS) is a single-gene neurodevelopmental disorder that is the most common inherited cause of intellectual disability in males. Its behavioral phenotype includes social avoidance and anxiety, language impairment, stereotypic and self-injurious behaviors, attentional deficits, hypersensitivity to sensory stimuli, and aggression.[1-4] Fragile X syndrome results from transcriptional silencing of the *FMR1* gene (OMIM 300624), leading to a failure to produce the fragile X mental retardation protein (FMRP). In the brain, the loss of FMRP impedes neural plasticity through dysregulation of messenger RNA translation.[5]

In addition to well-characterized effects on dendritic spine growth and plasticity,[6,7] a loss of FMRP also results in significant effects on the development of white matter through altered axon growth, refinement, and myelination.[8-10] These effects on white matter maturation may be particularly pronounced during the early postnatal period.[8,11] Altered axonal plasticity contributes to network dysfunction by affecting the development of local and global connectivity as well as functional specialization.[12] In *Fmr1* knockout model mice, there is converging evidence from multimodal imaging that both structural and functional connectivity is disrupted, with local connections favored vs long-range connections.[13]

Informed by findings from animal models, structural neuroimaging studies of toddlers and preschool-age children with FXS have reported atypical cortical gray matter and subcortical white matter volumes[14-17] and growth trajectories.[18] White matter in temporal regions[14] show the largest lobar differences in children with FXS relative to typically developing and developmentally delayed controls. Complementing these volumetric findings in white matter are reports of widespread alterations in functional connectivity in individuals with FXS.[19,20] However, only a handful of studies have used diffusion tensor imaging (DTI) to characterize the microstructural properties of white matter in individuals with FXS, and most of these studies have focused on later childhood and adolescence.[21-24] Although findings from this limited body of research have been somewhat inconsistent, they provide further evidence that white matter connectivity is fundamentally altered in individuals with FXS.

To our knowledge, there are no published data to date on the development of white matter fiber tracts in FXS during infancy. It is during this period that the clinical phenotype of FXS is typically first observed, when expression of FMRP is most crucial to the development of neural architecture,[8] and when postnatal alterations in axonal plasticity[25] may affect connectivity and functional specialization. Our aims in this study were to characterize the development of white matter using DTI in a longitudinal sample of infants with and without FXS, with the hypothesis that fractional anisotropy (FA) values would be lower in infants with FXS compared with control infants, and to perform a preliminary investigation into the association of structural connectivity with cognitive and behavioral development in infants with FXS.

Key Points

Question Is white matter development altered in infants with fragile X syndrome?

Findings In this longitudinal imaging study of 27 infants with fragile X syndrome and 73 typically developing control infants, 12 of 19 major white matter tracts investigated were significantly diminished in infants with fragile X syndrome compared with controls.

Meaning The effects of fragile X gene expression on the early development of white matter structural connectivity are well established at 6 months of age.

Methods

Participants

This study includes data from a longitudinal study of infants with FXS. Participants included 27 infants with FXS and 73 control infants. Full-mutation FXS (>200 CGG repeats) was confirmed via medical records, genetic testing (by polymerase chain reaction and Southern blot [eTable 1 in the Supplement]), or in a limited number of cases by parent report (n = 2). Infants in the control group had no first- or second-degree relatives with intellectual or psychiatric disorders, including FXS and autism spectrum disorder (ASD).[26] Further information on ascertainment strategies and exclusionary criteria can be found in the eAppendix in the Supplement. Parents provided written informed consent prior to participation. Procedures were approved by the Institutional Review Boards of the University of North Carolina at Chapel Hill and Washington University in St Louis.

Data were collected between August 1, 2008, and June 14, 2016, at the University of North Carolina, Chapel Hill and Washington University in St Louis. Infants and their families were enrolled and assessed when the infants were aged 6 months, with follow-up assessments at ages 12 and 24 months.

Clinical Measures

Cognitive Skills

Cognitive development was measured when the infants were aged 12 months using standard scores from the Mullen Scales of Early Learning,[27] a normed developmental assessment applicable to children from birth through 68 months. Nonverbal developmental quotients (NVDQs) were calculated from the visual reception and fine motor subscales, and verbal developmental quotients were calculated from the receptive and expressive language subscales.

Diffusion Tensor–Magnetic Resonance Imaging Data Acquisition, Processing, and Fiber Tractography

Pediatric imaging was completed during natural sleep at each clinical site using identical 3.0-T Siemens MAGNETOM Trio scanners (Siemens Medical Solutions). Infant-specific protocols were developed to be age appropriate and were standardized across sites with ongoing human and phantom

calibration.[28] The eAppendix in the Supplement contains information on DTI acquisition, preprocessing, quality control procedures, and diffusion measures. All corresponding processing tools are publicly available as part of the University of North Carolina at Chapel Heill–Utah National Alliance for Medical Imaging Computing DTI fiber tract analysis framework (https://www.nitrc.org/projects/namicdtifiber).[29]

Nineteen white matter fiber tracts were generated using seed label maps in the combined atlas space in 3D Slicer according to existing tractography methods (https://www.slicer.org).[29-31] Label maps were created for the following bilateral fiber tracts: inferior longitudinal fasciculus, uncinate fasciculus, anterior thalamic radiation, superior cerebellar peduncles (SCPs), and anterior and posterior limbs of the internal capsule. Label maps were also created for the middle cerebellar peduncles and 6 segments of the corpus callosum based on the Hofer and Frahm[32] anatomical parcellation method. This method yields corpus callosum segments that project to prefrontal regions (section I), the premotor and supplementary motor cortex (section II), the primary motor cortex (section III), the somatosensory cortex (section IV), and the parietal, temporal, and occipital regions (section Va and Vb). Spurious, incomplete, or anatomically incorrect fibers were removed via FiberViewerLight (University of North Carolina at Chapel Hill Neuro Image Research and Analysis Laboratory). Fiber profiles of FA, axial diffusivity (AD), and radial diffusivity (RD) were computed and averaged along each fiber tract.

Statistical Analysis

As of June 1, 2017, data were available for 100 infants who completed at least 1 DTI scan that passed quality control procedures (27 in the FXS group and 73 in the control group). All analyses were performed using SAS statistical software, version 9.3 (SAS Institute Inc). $P < .05$ (2-sided) was considered significant.

Longitudinal FA was examined across 3 visits (at age 6, 12, and 24 months) using general linear mixed models. For each model, mean FA in a given fiber tract was the dependent variable and fixed effects for the model included age, group, and group × age interaction. Clinical data collection site was included a priori as a control variable in all models to account for potential site differences in acquired magnetic resonance imaging data. The intercept term was treated as a random effect with the objective to reduce individual-to-individual variation. A false discovery rate procedure was used to correct for multiple comparisons, with adjusted P values presented as q values. Percentage differences in model-adjusted FA across all time points are reported relative to the control group.

Model fitting procedures were conducted to determine if sex of the infant or maternal education should be included as covariates. The 2 variables were assessed separately using a 2-step process. The first step included adding the potential covariate as a fixed effect and the second step included adding an interaction term with group (sex × group or maternal education × group). For both potential covariates across both steps, the base model demonstrated better model fit (based on lower Akaike information criterion scores). As such, these terms were not included in the final model reported in the main text. The

results for models including effects for sex and a sex × group interaction are reported in eTable 2 in the Supplement, while the results for models with the sex ratios matched in the FXS and control groups can be found in eTable 3 in the Supplement.

Secondary analyses investigated group differences in longitudinal RD and AD. These general linear mixed models included mean RD or AD in a given fiber tract as the dependent variable, and fixed effects included age, group, clinical data collection site, and group × age interaction.

Last, exploratory brain × behavior analyses were conducted within the FXS group. Analyses focused on tracts for which infants with FXS had significantly lower FA than infants in the control group. Linear regression was used to determine if 12-month FA in selected tracts was associated with 12-month Mullen Scales of Early Learning NVDQ and verbal developmental quotient scores, corrected for multiple comparisons. The 12-month imaging time point was selected, as this was the time point with the most magnetic resonance imaging data in infants with FXS (n = 18).

Results

Participant Characteristics

The 2 groups did not differ by proportion of males and females ($\chi^2 = 3.08$; $P = .07$) or by racial composition as defined by the parent ($\chi^2 = 1.95$; $P = .74$). The control group had mothers with higher levels of educational attainment compared with the FXS group ($\chi^2 = 16.63$; $P < .001$). **Table 1** includes additional participant demographic information as well as information on the number of scans at each time point (eTables 4 and 5 in the Supplement contain detailed information on data ascertainment).

Longitudinal Brain Development of White Matter Tracts

We examined FA in major white matter pathways across the brain at ages 6, 12, and 24 months, comparing development in the FXS and control groups. **Table 2** presents the full fixed effect results. In the left and right anterior limb of internal capsule, inferior longitudinal fasciculus, uncinate fasciculus, SCP (**Figure 1**), and sections I to III and Va of the corpus callosum (eFigure 1 in the Supplement), there were significant main effects for group, with lower FA in the FXS group compared with controls. There were no significant age × group interaction effects in any of these tracts, indicating that group differences in FA relative to controls did not significantly change over time. Compared with controls, infants with FXS had lower FA by a mean of 3.5% to 7.9% across these tracts. There were no significant effects for the midcerebellar peduncle, posterior limb of internal capsule, anterior thalamic radiation, and sections IV and Vb of the corpus callosum (eFigure 2 in the Supplement).

Results for secondary analyses of RD and AD can be found in eTables 6 and 7 in the Supplement. There were significant group main effects for RD in the left and right SCP, left anterior limb of internal capsule, left inferior longitudinal fasciculus, left uncinate fasciculus, and sections I, II, and Va of the corpus callosum (eFigure 3 in the Supplement). In these tracts, the FXS group had higher RD than the control group. There

Table 1. Sample Characteristics by Group

Characteristic	FXS (n = 27)	Control (n = 73)	Test Statistic	P Value
Longitudinal visit complement, No.				
At 6-mo visit	14	68	NA	NA
At 12-mo visit	18	50	NA	NA
At 24-mo visit	10	46	NA	NA
Age at 6-mo visit, mean (SD), mo	6.5 (0.8)	6.7 (0.7)	$t_{80} = -1.05$	.29
Age at 12-mo visit, mean (SD), mo	12.6 (0.8)	12.6 (0.6)	$t_{80} = 0.13$	.90
Age at 24-mo visit, mean (SD), mo	24.7 (1.0)	24.8 (1.3)	$t_{80} = -0.20$	.83
Male sex, No. (%)	22 (81.5)	46 (63.0)	$\chi^2 = 3.08$	.07
Child race, No. (%)				
White	22 (81.5)	59 (80.8)		
African American	0	3 (4.1)		
Asian	0	1 (1.4)	$\chi^2 = 1.95$	.74
>1 Race/ethnicity	3 (11.1)	7 (9.6)		
Not answered	2 (7.4)	3 (4.1)		
Maternal educational level, No. (%)				
High school diploma	10 (37.0)	10 (13.7)		
College degree	14 (51.9)	30 (41.1)	$\chi^2 = 16.63$	<.001
Graduate degree	2 (7.4)	33 (45.2)		
Missing	1 (3.7)	0		

Abbreviations: FXS, fragile X syndrome; NA, not applicable.

Table 2. Tests of Fixed Effects for Longitudinal Fractional Anisotropy Analyses[a]

Dependent Variable	Age		Diagnostic Group				Age × Group		
	F Score	P Value	F Score	P Value	q Value[b]	β (SE)	F Score	P Value	Decrease, %[c]
Left ALIC	224.18	<.001	13.68	<.001	.002	−.028 (.007)	0.14	.71	−6.25
Right ALIC	190.82	<.001	7.97	.008	.01	−.021 (.008)	0.74	.39	−6.69
Left PLIC	225.12	<.001	3.61	.06	.09	−.015 (.008)	0.01	.91	−3.01
Right PLIC	187.69	<.001	2.82	.10	.12	−.014 (.008)	0.11	.74	−3.42
Left ATR	172.07	<.001	4.36	.04	.06	−.018 (.008)	0.80	.37	−2.58
Right ATR	157.54	<.001	2.60	.11	.13	−.014 (.009)	0.01	.94	−3.18
Left ILF	276.87	<.001	16.90	<.001	.001	−.029 (.007)	0.03	.87	−7.44
Right ILF	524.64	<.001	19.87	<.001	.001	−.028 (.006)	1.69	.20	−5.14
Left uncinate	255.94	<.001	10.85	.002	.005	−.019 (.006)	0.68	.41	−3.84
Right uncinate	278.16	<.001	9.53	.004	.008	−.016 (.005)	0.58	.45	−3.45
Left SCP	129.13	<.001	15.48	<.001	.002	−.029 (.007)	0.27	.60	−6.91
Right SCP	162.09	<.001	24.00	<.001	.001	−.038 (.008)	1.13	.29	−7.92
MCP	137.01	<.001	3.31	.07	.09	−.020 (.011)	0.03	.85	−3.76
Corpus callosum									
Section I	510.48	<.001	19.98	<.001	.001	−.035 (.008)	0.17	.68	−7.59
Section II	471.95	<.001	14.75	<.001	.002	−.032 (.008)	0.18	.67	−6.96
Section III	333.09	<.001	6.42	.01	.02	−.025 (.010)	1.61	.21	−7.77
Section IV	153.25	<.001	2.23	.14	.15	−.016 (.010)	2.54	.12	−6.73
Section Va	231.19	<.001	5.62	.02	.03	−.020 (.008)	0.34	.56	−5.62
Section Vb	154.42	<.001	1.18	.28	.28	−.013 (.012)	0.75	.39	−4.20

Abbreviations: ALIC, anterior limb of internal capsule; ATR, anterior thalamic radiation; ILF, inferior longitudinal fasciculus; MCP, midcerebellar peduncle; PLIC, posterior limb of internal capsule; SCP, superior cerebellar peduncle.

[a] Clinical site included as a model covariate.

[b] The q Values are false discovery rate–corrected P values.

[c] Percentage decrease compares least squares means in fractional anisotropy across all time points in patients with fragile X syndrome compared with controls.

were no significant group main effects for AD and there were no significant age × group effects for RD or AD.

Associations With Behavior

Exploratory brain × behavior analyses within the FXS group focused on tracts for which infants with FXS had significantly lower FA than control infants. Results indicated a posi-

tive association between 12-month Mullen Scales of Early Learning NVDQ and 12-month FA in the left and right uncinate fasciculi (**Figure 2** and **Table 3**). Follow-up analyses of 12-month uncinate FA and 24-month NVDQ scores are presented in the eAppendix in the Supplement.

We next conducted 2 final tests to determine if these results were driven by the visual reception or fine motor com-

Figure 1. Fractional Anisotropy (FA) Values in Infants With Fragile X Syndrome (FXS)

A, Infants with FXS have lower FA values than controls in the bilateral anterior limb of the internal capsule (left: percentage difference, –6.25%; q = .002; right: percentage difference, –6.69%; q = .02). B, Infants with FXS have lower FA values than controls in the inferior longitudinal fasciculi (left: percentage difference, –7.44%; q = .001; right: percentage difference, –5.14%; q = .001). C, Infants with FXS have lower FA values than controls in the superior cerebellar peduncles (left: percentage difference, –6.91%; q = .002; right: percentage difference, –7.92%; q = .001). D, Infants with FXS have lower FA values than controls in the uncinate fasciculi (left: percentage difference, –3.84%; q = .005; right: percentage difference, –3.45%; q = .008). Error bars are ±1 SEM. q Values are false discovery rate–corrected P values for the group main effect. Percentage decrease compares least squares means in FA across all time points in patients with FXS compared with controls.

ponents of the NVDQ. Results for the left uncinate fasciculus indicated that visual reception and fine motor together explained 44.0% of the variance (adjusted R^2 = 0.366; $F_{2,15}$ = 5.91; P = .01). Visual reception was significantly associated with FA (β = 5.40 × 10^{-4}; P = .03); however, the fine motor component was not significantly associated with FA (β = 5.33 × 10^{-5}; P = .79). A similar pattern emerged for the right uncinate fasciculus; the visual reception and fine motor components together explained 60.7% of the variance (adjusted R^2 = 0.555; $F_{2,15}$ = 11.62; P < .001). The visual reception component was significantly associated with FA (β = 4.31 × 10^{-4}; P = .01); however, the fine motor component was not significantly associated with FA (β = 1.38 × 10^{-4}; P = .32). Nonverbal developmental quotients were not significantly associated with FA in any

other tracts, nor was 12-month verbal developmental quotient significantly correlated with FA in any tract (Table 3).

Discussion

In this longitudinal DTI study, we identified significant differences in the development of 12 of 19 white matter fiber pathways among infants with FXS. These differences were uniformly characterized by lower FA during the 6- to 24-month age interval, relative to typically developing infants in the control group. To our knowledge, these findings are the first to substantiate in human infants findings from nonhuman animal model studies concerning the essential role

Figure 2. Association Between 12-Month Mullen Scales of Early Learning (MSEL) Nonverbal Cognitive Skills (Nonverbal Developmental Quotient [NVDQ]) and 12-Month Fractional Anisotropy (FA) Values

A, Left uncinate fasciculus (adjusted $R^2 = 0.35$; $q = .04$). B, Right uncinate fasciculus (adjusted $R^2 = 0.55$; $q = .004$).

Table 3. Associations Between 12-Month FA and 12-Month MSEL NVDQ and VDQ

Characteristic	MSEL NVDQ				MSEL VDQ		
	F Score	P Value	q Value[a]	Adjusted R^2	F Score	P Value	Adjusted R^2
Left ALIC	3.45	.08	.16	0.125	0.76	.39	−0.014
Right ALIC	2.3	.14	.22	0.071	1.6	.22	0.033
Left ILF	1.32	.26	.35	0.076	0	.97	−0.062
Right ILF	0.21	.65	.65	−0.048	0.19	.67	−0.05
Left uncinate	10.06	.005	.03	0.347	0.04	.84	−0.06
Right uncinate	21.87	<.001	.004	0.551	0.16	.69	−0.051
Left SCP	0.46	.50	.55	−0.033	0.33	.57	−0.041
Right SCP	0.47	.50	.55	−0.032	0.39	.53	−0.036
Corpus callosum							
Section I	5.34	.03	.1	0.213	0.48	.49	−0.033
Section II	4.32	.05	.13	0.163	0.3	.58	−0.042
Section III	6.81	.01	.07	0.254	0.92	.35	−0.005
Section Va	3.1	.09	.16	0.109	1.31	.27	0.017

Abbreviations: ALIC, anterior limb of internal capsule; FA, fractional anisotropy; ILF, inferior longitudinal fasciculus; MSEL, Mullen Scales of Early Learning; NVDQ, Nonverbal Developmental Quotient; SCP, superior cerebellar peduncle;

VDQ, Verbal Developmental Quotient.

[a] The q Values are false discovery rate–corrected P values.

of FMRP in the early development of white matter connectivity.[8-10] Furthermore, our findings suggest that alterations to white matter structure in FXS are well established and relatively stable from age 6 months, 2 to 3 years prior to the mean age of diagnosis.[33] The white matter development observed in infants with FXS in our study appear to be distinct from those reported in similar studies of nonsyndromic ASD.[26,34,35] Infants and toddlers with ASD are reported to show higher initial FA followed by a period of relatively slower white matter development thereafter.[26,34] This finding is in contrast with the low and stable FA we observed in infants with FXS, and is consistent with previous work indicating that the neural signature of FXS may be distinct from that of idiopathic ASD.[14-16,36]

We observed some congruity in the white matter tracts showing significantly lower FA in infants with FXS. One set of pathways is predominantly involved in connectivity between subcortical regions—including the thalamus, basal ganglia, and cerebellum—and the prefrontal cortex (ie, bilateral SCP and anterior limb of internal capsule). Along with corpus callosum tracts linking primary and premotor cortices (corpus cal-

losum sections II-III), these pathways support the execution and control of motor function and support specific brain structures and behaviors known to be altered in FXS.[15,16,36] We also identified differences in the bilateral uncinate and inferior longitudinal fasciculi. The bilateral uncinate fasciculus connects the temporal lobe with the prefrontal lobe, and the inferior longitudinal fasciculus connects the temporal lobe with the occipital lobe, and together may constitute an indirect frontal-occipital circuit.[37] These pathways and the regions they connect have been implicated in previous diffusion tensor and structural imaging studies of older individuals with FXS,[23,38] and may contribute to aspects of the FXS phenotype associated with anxiety, language, and social-emotional functioning. This pattern of results suggests that loss of FMRP leads to variable outcomes on white matter circuitry, consistent with evidence that the protein is expressed differentially across brain regions and over time[39] and is pronounced in subcortical sensory and motor cells.[40]

Findings of lower FA in infants with FXS, together with secondary analyses indicating elevated radial diffusivity, may be attributed to underlying differences in structural connectiv-

ity associated with altered axon growth, refinement, and my-elination—all processes that are uniquely robust during infancy.[41,42] Although the effect of FMRP on synaptic plasticity has been the focus of extensive study,[43] considerably less is known about its effects on axon development or the interrelation of axonal and synaptic plasticity in its absence. Fragile X mental retardation protein is expressed by glial cells during early development and is necessary for the function of such cells.[44,45] Selective suppression of FMRP in glial cells only—in this case, astrocytes—is sufficient to bring about a neural and behavioral phenotype evocative of FXS in mice.[46] There is likewise evidence that the population of oligodendrocyte precursor cells is reduced in *Fmr1* knockout mice, and this reduction may contribute to subsequent myelin deficits arising during early postnatal development.[8] Moreover, FMRP is also necessary for regulating the growth and refinement of axons,[9,10] and axonal development absent of FMRP may induce dendritic spine dysmorphologic features.[9,10,47]

To explore the manner in which atypical white matter development may contribute to early functional deficits in children with FXS, we conducted a focused set of analyses into the association of FA with cognitive development at age 12 months. We found that the uncinate fasciculi were significantly positively associated with nonverbal developmental quotient. Motor function in FXS gradually diverges from a typical trajectory between infancy and toddlerhood[48] and atypical motor behaviors, such as stereotypies, are hallmark features of FXS from early in life.[4,49] We did not find that the SCP or other putative motor pathways were associated with NVDQ. Further analysis into this issue revealed that the visual reception component appeared to drive the association between uncinate FA and NVDQ. Although it is difficult to ascribe a particular function to the uncinate, particularly during infancy, it is possible that the brain-behavior association we observed pertains to its role in limbic system function, which may be reflected in visual reception scores.[50]

The results of this study highlight white matter as a potential target for early intervention. White matter undergoes robust development from infancy through early adulthood[51] and remains a highly plastic target for intervention throughout the lifespan.[25] There is evidence linking the early development of specific white matter regions to cognitive and behavioral features relevant to the FXS phenotype, including language, learning, and memory, as well as repetitive behaviors.[52-54] Although research into the effects of intervention on the structural properties of white matter are limited, there is some evidence that axonal circuitry is particularly sensitive to treatment effects, and that these may be quantified through magnetic resonance imaging approaches such as DTI.[55,56] However, most children with FXS are not diagnosed in the first year of life.[33] Achieving the goal of infant intervention for FXS, including intervention targeting early developing white matter, would likely require expanded efforts to screen newborns.[57,58]

Limitations

The results of this study should be considered in light of several limitations. Although DTI data describe the general structural properties of white matter, they cannot be used to pinpoint precise attributes, such as myelination or axonal density, contributing to observed values.[59] Comprehensive family pedigree and genetic data were not available for most infants in our study; thus, we cannot discuss associations of FA with FMRP levels. Follow-up studies should consider direct quantification of FMRP to expand on the present findings. We plan to continue following up these children beyond infancy, when the full FXS behavioral phenotype can be assessed. Likewise, in later childhood symptoms of ASD may be more accurately and reliably assessed, to determine what effect, if any, a comorbid diagnosis of ASD has on the early development of structural connectivity. Additional studies are also warranted to further elucidate how the development of neural circuitry is associated with cognitive and behavioral development, particularly beyond the age of 12 months when more stable estimates may be ascertained. This includes more fine-grained cognitive measures, as well as other aspects of the FXS behavioral phenotype including social deficits, hyperactivity, anxiety, and repetitive behavior. Finally, while this study offers new insights into brain development in infants with FXS, replication with a larger number of participants and repeated measures is necessary to more accurately characterize developmental trajectories.

Conclusions

In this longitudinal DTI study of infants with and without FXS, we identified evidence of diminished development of structural connectivity in comparison with typically developing infants. In general, white matter circuits showing the largest alterations are integral to subcortical and cortical motor regions as well as temporal cortical connectivity. To our knowledge, this is the first brain imaging study of children with FXS during infancy, the results of which suggest that the neurobiological effects of FMRP loss are strongly established well in advance of the mean age of diagnosis and during a time when the first behavioral features emerge.[33]

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REFERENCES

1. Finestack LH, Richmond EK, Abbeduto L. Language development in individuals with fragile X syndrome. *Top Lang Disord*. 2009;29(2):133-148.

2. Kau ASM, Reider EE, Payne L, Meyer WA, Freund L. Early behavior signs of psychiatric phenotypes in fragile X syndrome. *Am J Ment Retard*. 2000;105(4):286-299.

3. Sullivan K, Hatton D, Hammer J, et al. ADHD symptoms in children with FXS. *Am J Med Genet A*. 2006;140(21):2275-2288.

4. Wolff JJ, Bodfish JW, Hazlett HC, Lightbody AA, Reiss AL, Piven J. Evidence of a distinct behavioral phenotype in young boys with fragile X syndrome and autism. *J Am Acad Child Adolesc Psychiatry*. 2012;51(12):1324-1332.

5. Darnell JC, Klann E. The translation of translational control by FMRP: therapeutic targets for FXS. *Nat Neurosci*. 2013;16(11):1530-1536.

6. Comery TA, Harris JB, Willems PJ, et al. Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits. *Proc Natl Acad Sci U S A*. 1997;94(10):5401-5404.

7. Pan F, Aldridge GM, Greenough WT, Gan W-B. Dendritic spine instability and insensitivity to modulation by sensory experience in a mouse model of fragile X syndrome. *Proc Natl Acad Sci U S A*. 2010;107(41):17768-17773.

8. Pacey LKK, Xuan ICY, Guan S, et al. Delayed myelination in a mouse model of fragile X syndrome. *Hum Mol Genet*. 2013;22(19):3920-3930.

9. Bureau I, Shepherd GMG, Svoboda K. Circuit and plasticity defects in the developing somatosensory cortex of FMR1 knock-out mice. *J Neurosci*. 2008;28(20):5178-5188.

10. Tessier CR, Broadie K. Drosophila fragile X mental retardation protein developmentally regulates activity-dependent axon pruning. *Development*. 2008;135(8):1547-1557.

11. Pacey LKK, Doering LC. Developmental expression of FMRP in the astrocyte lineage: implications for fragile X syndrome. *Glia*. 2007;55(15):1601-1609.

12. Belmonte MK, Bourgeron T. Fragile X syndrome and autism at the intersection of genetic and neural networks. *Nat Neurosci*. 2006;9(10):1221-1225.

13. Haberl MG, Zerbi V, Veltien A, Ginger M, Heerschap A, Frick A. Structural-functional connectivity deficits of neocortical circuits in the Fmr1 (-/y) mouse model of autism. *Sci Adv*. 2015;1(10):e1500775.

14. Hazlett HC, Poe MD, Lightbody AA, et al. Trajectories of early brain volume development in fragile X syndrome and autism. *J Am Acad Child Adolesc Psychiatry*. 2012;51(9):921-933.

15. Hazlett HC, Poe MD, Lightbody AA, et al. Teasing apart the heterogeneity of autism: Same behavior, different brains in toddlers with fragile X syndrome and autism. *J Neurodev Disord*. 2009;1(1):81-90.

16. Hoeft F, Walter E, Lightbody AA, et al. Neuroanatomical differences in toddler boys with fragile x syndrome and idiopathic autism. *Arch Gen Psychiatry*. 2011;68(3):295-305.

17. Hoeft F, Lightbody AA, Hazlett HC, Patnaik S, Piven J, Reiss AL. Morphometric spatial patterns differentiating boys with fragile X syndrome, typically developing boys, and developmentally delayed boys aged 1 to 3 years. *Arch Gen Psychiatry*. 2008;65(9):1087-1097.

18. Hoeft F, Carter JC, Lightbody AA, Cody Hazlett H, Piven J, Reiss AL. Region-specific alterations in brain development in one- to three-year-old boys with fragile X syndrome. *Proc Natl Acad Sci U S A*. 2010;107(20):9335-9339.

19. Hall SS, Jiang H, Reiss AL, Greicius MD. Identifying large-scale brain networks in fragile X syndrome. *JAMA Psychiatry*. 2013;70(11):1215-1223.

20. Wang J, Ethridge LE, Mosconi MW, et al. A resting EEG study of neocortical hyperexcitability and altered functional connectivity in fragile X syndrome. *J Neurodev Disord*. 2017;9(1):11.

21. Villalon-Reina J, Jahanshad N, Beaton E, Toga AW, Thompson PM, Simon TJ. White matter microstructural abnormalities in girls with chromosome 22q11.2 deletion syndrome, fragile X or Turner syndrome as evidenced by diffusion tensor imaging. *Neuroimage*. 2013;81:441-454.

22. Barnea-Goraly N, Eliez S, Hedeus M, et al. White matter tract alterations in fragile X syndrome: preliminary evidence from diffusion tensor imaging. *Am J Med Genet B Neuropsychiatr Genet*. 2003;118B(1):81-88.

23. Hall SS, Dougherty RF, Reiss AL. Profiles of aberrant white matter microstructure in fragile X syndrome. *Neuroimage Clin*. 2016;11:133-138.

24. Haas BW, Barnea-Goraly N, Lightbody AA, et al. Early white-matter abnormalities of the ventral frontostriatal pathway in fragile X syndrome. *Dev Med Child Neurol*. 2009;51(8):593-599.

25. Fields RD. A new mechanism of nervous system plasticity: activity-dependent myelination. *Nat Rev Neurosci*. 2015;16(12):756-767.

26. Wolff JJ, Gu H, Gerig G, et al; IBIS Network. Differences in white matter fiber tract development present from 6 to 24 months in infants with autism. *Am J Psychiatry*. 2012;169(6):589-600.

27. Mullen EME. *Mullen Scales of Early Learning*. Circle Pines, MN: AGS; 1995.

28. Gouttard S, Styner M, Prastawa M, Piven J, Gerig G. Assessment of reliability of multi-site neuroimaging via traveling phantom study. In: Metaxas D, Axel L, Fichtinger G, Székely G, eds. *Medical Image Computing and Computer-Assisted Intervention–MICCAI 2008. Lecture Notes in Computer Science*. Vol 5242. Berlin, Germany: Springer; 2008:263-270.

29. Verde AR, Budin F, Berger J-B, et al. UNC-Utah NA-MIC framework for DTI fiber tract analysis. *Front Neuroinform*. 2014;7(51):51.

30. Catani M, Thiebaut de Schotten M. A diffusion tensor imaging tractography atlas for virtual in vivo dissections. *Cortex*. 2008;44(8):1105-1132.

31. Mori S, Wakana S, Van Zijl PCM, Nagae-Poetscher LM. *MRI Atlas of Human White Matter*. Amsterdam, The Netherlands: Elsevier Science Publishing Co; 2005.

32. Hofer S, Frahm J. Topography of the human corpus callosum revisited—comprehensive fiber

tractography using diffusion tensor magnetic resonance imaging. *Neuroimage*. 2006;32(3):989-994.

33. Bailey DB Jr, Raspa M, Bishop E, Holiday D. No change in the age of diagnosis for fragile X syndrome: findings from a national parent survey. *Pediatrics*. 2009;124(2):527-533.

34. Solso S, Xu R, Proudfoot J, et al. Diffusion tensor imaging provides evidence of possible axonal overconnectivity in frontal lobes in autism spectrum disorder toddlers. *Biol Psychiatry*. 2016; 79(8):676-684.

35. Conti E, Mitra J, Calderoni S, et al. Network over-connectivity differentiates autism spectrum disorder from other developmental disorders in toddlers: a diffusion MRI study. *Hum Brain Mapp*. 2017;38(5):2333-2344.

36. Wolff JJ, Hazlett HC, Lightbody AA, Reiss AL, Piven J. Repetitive and self-injurious behaviors: associations with caudate volume in autism and fragile X syndrome. *J Neurodev Disord*. 2013;5(1):12.

37. Wahl M, Li Y-O, Ng J, et al. Microstructural correlations of white matter tracts in the human brain. *Neuroimage*. 2010;51(2):531-541.

38. Gothelf D, Furfaro JA, Hoeft F, et al. Neuroanatomy of fragile X syndrome is associated with aberrant behavior and the fragile X mental retardation protein (FMRP). *Ann Neurol*. 2008;63 (1):40-51.

39. Hinds HL, Ashley CT, Sutcliffe JS, et al. Tissue specific expression of FMR-1 provides evidence for a functional role in fragile X syndrome. *Nat Genet*. 1993;3(1):36-43.

40. Zorio DAR, Jackson CM, Liu Y, Rubel EW, Wang Y. Cellular distribution of the fragile X mental retardation protein in the mouse brain. *J Comp Neurol*. 2017;525(4):818-849.

41. Klawiter EC, Schmidt RE, Trinkaus K, et al. Radial diffusivity predicts demyelination in ex vivo multiple sclerosis spinal cords. *Neuroimage*. 2011;55 (4):1454-1460.

42. Wolff JJ, Gerig G, Lewis JD, et al; IBIS Network. Altered corpus callosum morphology associated with autism over the first 2 years of life. *Brain*. 2015; 138(pt 7):2046-2058.

43. Sidorov MS, Auerbach BD, Bear MF. Fragile X mental retardation protein and synaptic plasticity. *Mol Brain*. 2013;6(1):15.

44. Jacobs S, Doering LC. Astrocytes prevent abnormal neuronal development in the fragile x mouse. *J Neurosci*. 2010;30(12):4508-4514.

45. Wang H, Ku L, Osterhout DJ, et al. Developmentally-programmed FMRP expression in oligodendrocytes: a potential role of FMRP in regulating translation in oligodendroglia progenitors. *Hum Mol Genet*. 2004;13(1):79-89.

46. Hodges JL, Yu X, Gilmore A, et al. Astrocytic contributions to synaptic and learning abnormalities in a mouse model of fragile X syndrome. *Biol Psychiatry*. 2017;82(2):139-149.

47. Antar LN, Li C, Zhang H, Carroll RC, Bassell GJ. Local functions for FMRP in axon growth cone motility and activity-dependent regulation of filopodia and spine synapses. *Mol Cell Neurosci*. 2006;32(1-2):37-48.

48. Roberts JE, McCary LM, Shinkareva SV, Bailey DB Jr. Infant development in fragile X syndrome: cross-syndrome comparisons. *J Autism Dev Disord*. 2016;46(6):2088-2099.

49. Hogan AL, Caravella KE, Ezell J, Rague L, Hills K, Roberts JE. Autism spectrum disorder symptoms in infants with fragile X syndrome: a prospective case series. *J Autism Dev Disord*. 2017;47(6):1628-1644.

50. Elison JT, Wolff JJ, Heimer DC, et al; IBIS Network. Frontolimbic neural circuitry at 6 months predicts individual differences in joint attention at 9 months. *Dev Sci*. 2013;16(2):186-197.

51. Westlye LT, Walhovd KB, Dale AM, et al. Life-span changes of the human brain white matter: diffusion tensor imaging (DTI) and volumetry. *Cereb Cortex*. 2010;20(9):2055-2068.

52. Swanson MR, Wolff JJ, Elison JT, et al. Splenium development and early spoken language in human infants [published online October 21, 2015]. *Dev Sci*. doi:10.1111/desc.12360

53. Wolff JJ, Swanson MR, Elison JT, et al; IBIS Network. Neural circuitry at age 6 months associated with later repetitive behavior and sensory responsiveness in autism. *Mol Autism*. 2017;8(1):8.

54. Short SJ, Elison JT, Goldman BD, et al. Associations between white matter microstructure and infants' working memory. *Neuroimage*. 2013;64 (0):156-166.

55. Hofstetter S, Friedmann N, Assaf Y. Rapid language-related plasticity: microstructural changes in the cortex after a short session of new word learning. *Brain Struct Funct*. 2017;222(3):1231-1241.

56. Scholz J, Klein MC, Behrens TEJ, Johansen-Berg H. Training induces changes in white-matter architecture. *Nat Neurosci*. 2009;12 (11):1370-1371.

57. Tassone F. Newborn screening for fragile X syndrome. *JAMA Neurol*. 2014;71(3):355-359.

58. Bailey DB Jr, Berry-Kravis E, Gane LW, et al. Fragile x newborn screening: lessons learned from a multisite screening study. *Pediatrics*. 2017;139(suppl 3):S216-S225.

59. Mori S, Zhang J. Principles of diffusion tensor imaging and its applications to basic neuroscience research. *Neuron*. 2006;51(5):527-539.