Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix. Methods and Results

Ascertainment Strategy.

Infants with fragile X syndrome were recruited using several strategies including: postings in fragile X list serves, family advocacy conferences, the UNC Carolina Institute of Developmental Disabilities Research Registry, and the Fragile X specialty clinic at WUSM. Infants in the control group were ascertained from the community as part of the Infant Brain Imaging Study (IBIS).

Additional Exclusionary Criteria.

Further exclusionary criteria for control infants included: significant medical conditions known to affect brain development, sensory impairment, low birth weight (< 2,200 g) or prematurity (<36 weeks gestation), perinatal brain injury secondary to birth complications or exposure to specific medication or neurotoxins during gestation, non-English speaking immediate family, contraindication for MRI, adoption, and first degree relative with psychosis, schizophrenia, or bipolar disorder.

Clinical Measures

Developmental quotients were used over *t*-scores from the MSEL[1] as 28% (*n*= 5) of the FXS infants had *t*-scores at floor at age 12-months.

Data Coordination.

Data coordination was managed by the Montreal Neurological Institute (MNI) at McGill University and neuroimaging data processing was performed at the University of North Carolina and the Scientific Computing and Imaging Institute at the University of Utah.

MRI Acquisition.

Pediatric imaging was completed during natural sleep at each clinical site using identical 3-T Siemens TIM Trio scanners (Siemens Medical Solutions, Malvern, Pa.) equipped with 12-channel head coils. The imaging protocol included (1) a localizer scan, (2) 3D T1 MPRAGE: TR=2400ms, TE=3.16ms, 160 sagittal slices, FOV=256, voxel size = $1mm^3$, (3) 3D T2 FSE TR=3200ms, TE=499ms, 160 sagittal slices, FOV=256, voxel size = $1mm^3$, and (4) a 25 direction diffusion tensor imaging (DTI) sequence. The DTI sequence was acquired as an ep2d_diff pulse sequence with a field of view of 190 mm (6 and 12 months) or 209 mm (24 months), 75–81 transversal slices, slice thickness of 2 mm isotropic, $2\times2\times2\text{-}mm^3$ voxel resolution, TR=12,800–13,300 ms, TE=102 ms, with 26 DWI volumes with b values between 0 and 1,000 s/mm^2 in increments of 40, including a single b = $0s/mm^2$, 25 gradient directions, and a scan time of 5–6 minutes.

A number of quality control procedures were employed to assess scanner stability and reliability across sites, time, and procedures. Geometric phantoms were scanned monthly and human phantoms (two adult subjects) were scanned annually to monitor scanner stability at each site across the study period. Details on the stability procedures for scanner quality control checks are described elsewhere [2].

Image Preprocessing.

Data from diffusion-weighted imaging were processed for appropriate quality via DTIprep [3,4], which automatically detects artifacts, corrects for motion and eddy current deformations, excludes diffusion weighted images with artifacts, and generates a full report. Expert raters manually removed additional images presenting with residual artifacts. Data sets with fewer than 18 (of 25 total) gradient diffusion-weighted images after this quality procedure were excluded from further processing due to a low signal-to-noise ratio and potential biases for fractional anisotropy assessment [5]. Diffusion tensor images were computed via weighted least squares estimation with additional mapping of invalid tensors (with negative Eigenvalues) to the nearest valid tensors using the DTIprocess software package (https://www.nitrc.org/projects/dtiprocess/). Diffusion-tensor imaging data for all infants (ages 6 to 24 months) were mapped into a common atlas space using a log-Euclidean tensor interpolation [6,7].

Across all FXS and control scans acquired, 84% resulted in a successful DTI acquisition that passed this quality control procedure. We quantified the number of gradients remaining for analyses after all quality control procedures and found that FXS and control infants did not differ in number of remaining gradients, $t(202) = -0.41$, $p = .68$. Out

of 26 total DWI volumes (25 gradients plus b = 0 s/mm^2), FXS infants had 24.80 remaining gradients (*SD* = 1.08, range 22-26), and control infants had 24.90 (*SD* = 1.34, range 20-26).

Diffusion Metrics.

Fiber profiles of fractional anisotropy (FA) values were computed and averaged along each fiber tract. Fractional anisotropy is an index measuring the degree of anisotropy of local diffusivity, ranging from 0 for isotropic diffusion, to 1 for strongly directional diffusivity 29. Fiber profiles of axial diffusivity (AD), an index of diffusion along the principal fiber direction, and radial diffusivity (RD), an index of diffusion orthogonal to the primary fiber direction, were also produced to inform FA results. FA was chosen as the primary outcome measure of defining fiber profiles because it models the association between the three eigenvalues and is thought to correspond to organizational properties that reflect development [8–11]. All corresponding processing tools are publicly available as part of the UNC-Utah NA-MIC DTI fiber tract analysis framework (www.nitrc.org/projects/namicdtifiber) [6].

Results.

To follow-up the exploratory brain-behavior analyses presented in the main results, we analyzed the relation of uncinate FA at 12 months with nonverbal developmental quotient assessed at age 24 months (n = 13). FA in the left uncinate at 12 months was not significantly associated with 24 month Nonverbal DQ (Adj.R^2 = -.039, $F(1,11)$= 0.55, p= .47), however FA in the right uncinate at 12-months was significantly associated with 24 month Nonverbal DQ at age 24 months (Adj.R^2 = .340, $F(1,11)$= 7.21, p= .02).

eTable 1. Number of CGG Repeats for Each Infant with Fragile X Syndrome

Case Number	Number of CGG repeats
1	full mutation (>200)
2	full mutation (>200)
3	full mutation (>200)
4	full mutation (>200)
5	full mutation (>200)
6	full mutation (>200); methylation mosaicism
7	full mutation (>200); methylation mosaicism
8	full mutation (>200); methylation mosaicism
9	full mutation (>200); methylation mosaicism
10	201
11	235
12	200-300
13	200-500
14	230-600
15	300
16	300-900
17	300-900
18	300-1100
19	400
20	500
21	500-700
22	700
23	700-900
24	900
25	>1000
26	full mutation (reported by parent)
27	full mutation (reported by parent)

eTable 2. Tests of Fixed Effects for Longitudinal Fractional Anisotropy Analyses With Sex of Child as a Covariate

	Age		Group		Age x Group		Sex		Sex x Group	
Dependent Variable	*F*	*P* Value	*F*	*P* Value	*F*	*P* Value	*F*	*P* Value	*F*	*P* Value
ALIC-L	222.80	<.001	12.91	.001	.0.16	.69	0.79	.38	0.28	.59
ALIC-R	192.86	<.001	6.09	.01	0.61	.44	0.01	.90	2.13	.15
PLIC-L	227.78	<.001	3.43	.07	0.00	.94	3.15	.08	0.26	.61
PLIC-R	191.32	<.001	1.86	.18	0.09	.76	0.34	.56	2.85	.11
ATR-L	167.77	<.001	4.88	.03	0.65	.42	1.04	.31	0.42	.52
ATR-R	157.54	<.001	1.99	.46	0.02	.89	0.05	.82	0.75	.39
ILF-L	260.90	<.001	19.48	<.001	0.15	.69	2.53	.12	1.53	.22
ILF-R	486.20	<.001	22.55	<.001	0.89	.35	2.29	.14	1.89	.17
Uncinate- L	239.29	<.001	11.81	.001	0.31	.57	2.84	.10	0.61	.44
Uncinate-R	267.72	<.001	9.79	.003	0.45	.50	0.16	.69	0.31	.58
SCP-L	128.85	<.001	14.87	<.001	0.30	.58	2.11	.15	0.71	.40
SCP-R	161.78	<.001	21.17	<.001	1.22	.27	0.12	.72	0.79	.37
MCP	137.50	<.001	2.45	.12	0.04	.84	0.59	.44	1.31	.25
CC- section I	503.01	<.001	21.12	<.001	0.15	.70	0.18	.68	1.60	.21
CC- section II	468.22	<.001	11.67	.001	0.22	.64	0.38	.54	0.17	.68
CC- section III	331.92	<.001	4.30	.04	1.63	.21	0.50	.48	0.47	.49
CC- section IV	151.94	<.001	2.05	.16	2.56	.11	0.76	.38	0.02	.88
CC- section Va	230.75	<.001	5.07	.03	0.35	.55	0.42	.52	0.30	.58
CC- section Vb	154.73	<.001	0.88	.35	0.84	.36	1.12	.29	0.09	.76

eTable 3. Tests of Fixed Effects for Longitudinal Fractional Anisotropy Analyses With Sex Ratios Matched in FXS and Control Groups

	Age		Diagnostic Group			Age x Group	
Dependent Variable	*F*	*P* Value	*F*	*P* Value	*q-value*	*F*	*P* Value
ALIC-L	205.58	<.001	16.06	<.001	.001	0.22	.63
ALIC-R	177.20	<.001	10.62	.003	.006	0.42	.52
PLIC-L	197.33	<.001	5.13	.03	.051	0.12	.73
PLIC-R	170.73	<.001	4.56	.04	.061	0.00	.99
ATR-L	151.37	<.001	4.11	.05	.072	0.82	.37
ATR-R	143.37	<.001	2.89	.10	.113	0.02	.88
ILF-L	254.33	<.001	15.57	<.001	.001	0.00	.98
ILF-R	465.81	<.001	16.94	<.001	.001	1.82	.18
Uncinate- L	254.20	<.001	11.88	.001	.004	1.22	.27
Uncinate-R	252.65	<.001	9.08	.005	.010	0.79	.38
SCP-L	126.34	<.001	17.27	<.001	.001	0.38	.54
SCP-R	169.02	<.001	28.97	<.001	.001	1.82	.18
MCP	123.28	<.001	3.64	.06	.083	0.05	.82
CC- sec. I	501.75	<.001	17.95	<.001	.001	0.19	.66
CC- sec. II	434.93	<.001	12.13	.001	.004	0.17	.68
CC- sec. III	293.00	<.001	5.10	.03	.051	1.84	.18
CC- sec. IV	156.62	<.001	1.89	.18	.190	3.99	.05
CC- sec. Va	231.56	<.001	3.57	.06	.083	0.71	.40
CC- sec. Vb	155.88	<.001	0.69	.41	.412	0.73	.40

eTable 4. Number of Infants at Each of the Three Possible Time Points, by Group and Sex of the Infant

	FXS M/F (%M)	Control M/F (%M)	χ^2 test
6-month visit	9/5 (64%)	42/26 (61%)	$X^2 = 0.03$, $p = .85$
12-month visit	15/3 (83%)	33/17 (66%)	$X^2 = 1.91$, $p = .16$
24-month visit	7/3 (70%)	29/17 (63%)	$X^2 = 0.17$, $p = .67$

eTable 5. Longitudinal Scan Complement Indicating Number of Infants with Cross-Sectional and Longitudinal Data, by Group and Sex of the Infant

MRI time point(s)	FXS	Control
6-month only	4 (3M, 1F)	10 (5M, 5F)
12-month only	7 (7M, 0F)	0
24-month only	3 (3M, 0F)	2 (1M, 1F)
6- and 12-months	6 (5M, 1F)	17 (12M, 5F)
6- and 24-months	2 (1M, 1F)	10 (7M, 3F)
12- and 24-months	3 (3M, 0F)	4 (3M, 1F)
6-, 12-, and 24-months	2 (0M, 2F)	30 (18M, 12F)

eTable 6. Tests of Fixed Effects for Longitudinal Radial Diffusivity Analyses, Clinical Site Included as a Model Covariate

	Age		Diagnostic Group				Age x Group		
Dependent Variable	*F*	*P* Value	*F*	*P* Value	*q-value*	*β (SE)*	*F*	*P* Value	Percent Increase
ALIC-L	110.14	<.001	10.74	.002	.01	4.30E-05 (1.30E-05)	0.33	.57	5.22
ALIC-R	130.40	<.001	3.91	.05	.11	2.20E-05 (1.10E-05)	0.81	.37	4.89
PLIC-L	223.83	<.001	0.33	.56	.63	7.47E-06 (1.30E-05)	0.13	.71	1.70
PLIC-R	151.59	<.001	0.43	.51	.63	8.58E-06 (1.30E-05)	0.53	.46	2.67
ATR-L	176.88	<.001	0.38	.54	.63	7.73E-06 (1.30E-05)	0.00	.99	1.09
ATR-R	159.49	<.001	0.50	.82	.82	-2.81E-06 (1.20E-05)	0.79	.38	1.09
ILF-L	381.66	<.001	10.59	.002	.01	5.80E-05 (1.80E-05)	1.11	.30	5.73
ILF-R	367.33	<.001	1.80	.18	.30	2.40E-05 (1.80E-05)	0.09	.76	2.64
Uncinate- L	422.24	<.001	6.55	.01	.04	2.60E-05 (1.00E-05)	0.90	.34	2.03
Uncinate-R	364.60	<.001	2.64	.11	.21	1.70E-05 (1.00E-05)	0.28	.59	1.53
SCP-L	30.49	<.001	5.97	.02	.04	6.70E-05 (2.70E-05)	0.00	.97	7.93
SCP-R	37.03	<.001	13.15	.001	.01	1.02E-04 (2.80E-05)	1.24	.27	9.14
MCP	81.24	<.001	0.14	.70	.74	-1.00E-05 (3.00E-05)	2.13	.15	2.90
CC- sec. I	557.67	<.001	11.20	.002	.01	4.80E-05 (1.40E-05)	0.58	.45	5.31
CC- sec. II	521.64	<.001	9.73	.003	.01	5.20E-05 (1.70E-05)	0.28	.60	5.91
CC- sec. III	311.65	<.001	2.44	.12	.22	3.50E-05 (2.20E-05)	0.95	.33	6.62
CC- sec. IV	218.25	<.001	0.87	.35	.48	1.90E-05 (2.00E-05)	1.84	.18	5.07
CC- sec. Va	275.56	<.001	7.16	.01	.03	3.80E-05 (1.40E-05)	0.03	.86	5.42
CC- sec. Vb	141.11	<.001	1.55	.22	.32	3.10E-05 (2.50E-05)	0.56	.45	5.94

Note: Model covariates include clinical data collection site. *q*-values are FDR corrected *P* values. Percent increase compares least squares means in RD across all time points in FXS compared to controls.

eTable 7. Tests of Fixed Effects for Longitudinal Axial Diffusivity Analyses, Clinical Site Included as a Model Covariate

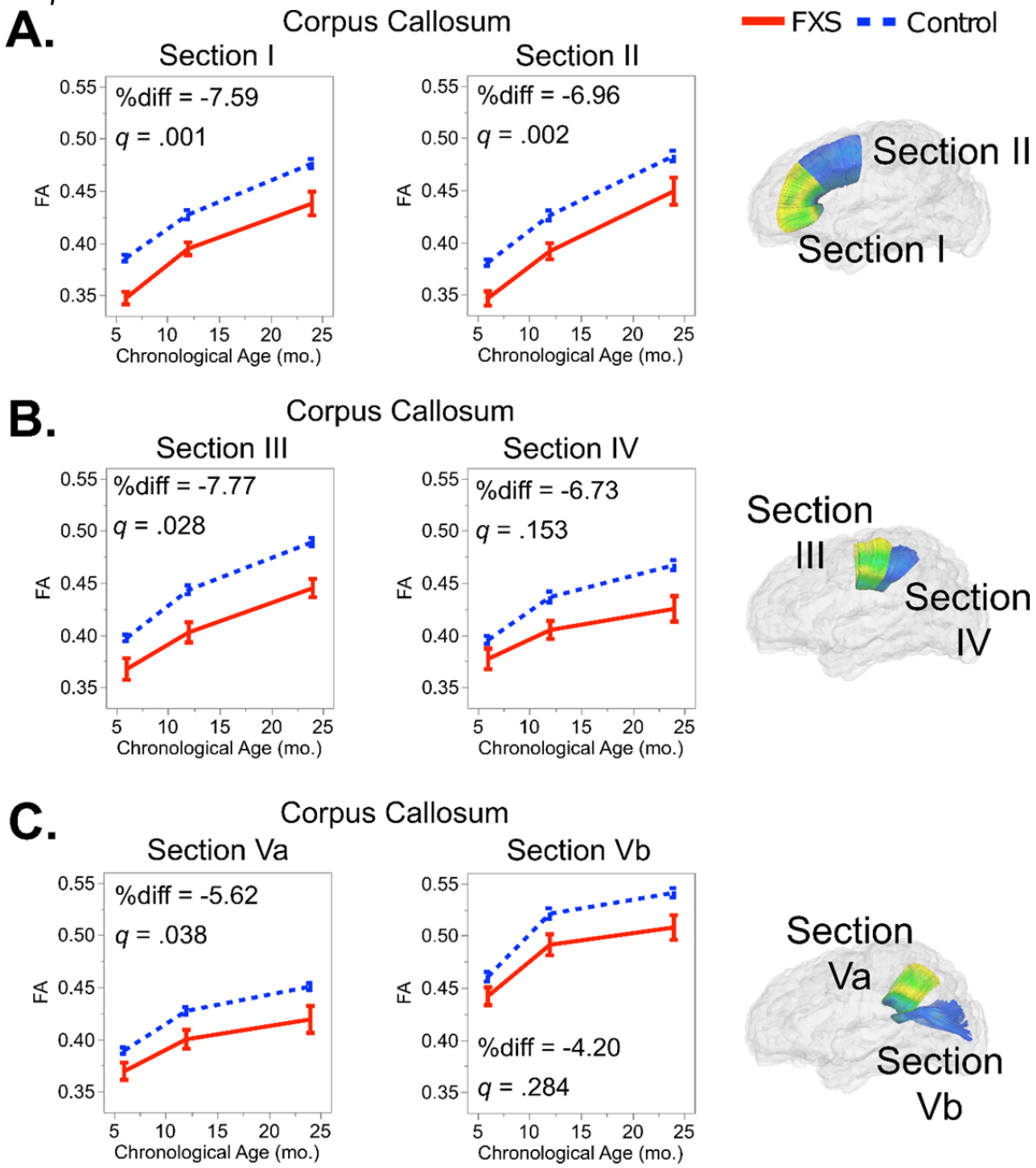
	Age		Diagnostic Group				Age x Group		
Dependent Variable	*F*	*P* Value	*F*	*P* Value	*q-value*	*β (SE)*	*F*	*P* Value	Percent Increase/Decrease
ALIC-L	0.00	.99	0.89	.35	.73	1.80E-05 (1.90E-05)	0.27	.60	0.69
ALIC-R	0.12	.72	0.16	.69	.82	-5.32E-06 (1.30E-05)	0.04	.83	-0.70
PLIC-L	10.86	.001	0.76	.38	.73	-1.00E-05 (1.50E-05)	0.00	.95	-0.92
PLIC-R	5.73	.01	0.42	.52	.73	-9.88E-06 (1.50E-05)	0.00	.97	-0.72
ATR-L	29.30	<.001	3.38	.07	.65	-3.00E-05 (1.40E-05)	0.43	.51	-1.27
ATR-R	27.15	<.001	5.97	.02	.38	-4.00E-05 (1.50E-05)	0.82	.37	-1.98
ILF-L	94.91	<.001	0.48	.49	.73	2.20E-05 (3.10E-05)	2.55	.12	-0.47
ILF-R	48.61	<.001	0.12	.73	.82	-1.00E-05 (3.20E-05)	0.22	.64	-1.39
Uncinate- L	113.86	<.001	1.71	.20	.73	-2.00E-05 (1.40E-05)	0.28	.59	-0.94
Uncinate-R	92.85	<.001	2.80	.10	.65	-2.00E-05 (1.30E-05)	0.28	.59	-1.26
SCP-L	0.01	.90	0.76	.38	.73	4.60E-05 (5.20E-05)	0.20	.66	3.94
SCP-R	0.00	.96	2.02	.16	.73	7.00E-05 (5.00E-05)	0.15	.70	3.41
MCP	12.44	<.001	0.77	.38	.73	-4.00E-05 (4.50E-05)	1.12	.29	-0.64
CC- sec. I	130.02	<.001	0.32	.57	.73	-8.30E-06 (1.50E-05)	0.47	.49	-1.13

CC- sec. II	134.90	<.001	0.00	.95	.95	1.13E-06 (1.90E-05)	0.07	.79	-0.26
CC- sec. III	74.58	<.001	0.31	.58	.73	-1.00E-05 (2.60E-05)	0.00	.97	-0.92
CC- sec. IV	82.27	<.001	0.06	.80	.85	-5.05E-06 (2.10E-05)	0.16	.69	-0.81
CC- sec. Va	137.77	<.001	0.63	.43	.73	1.50E-05 (1.80E-05)	0.05	.83	0.80
CC- sec. Vb	14.86	<.001	1.20	.28	.73	3.40E-05 (3.10E-05)	0.15	.70	1.57

Note: Model covariates include clinical data collection site. *q*-values not shown as no *P* values < .05. Percent increase (positive numbers), and decrease (negative numbers) compares least squares means in AD across all time points in FXS compared to controls.

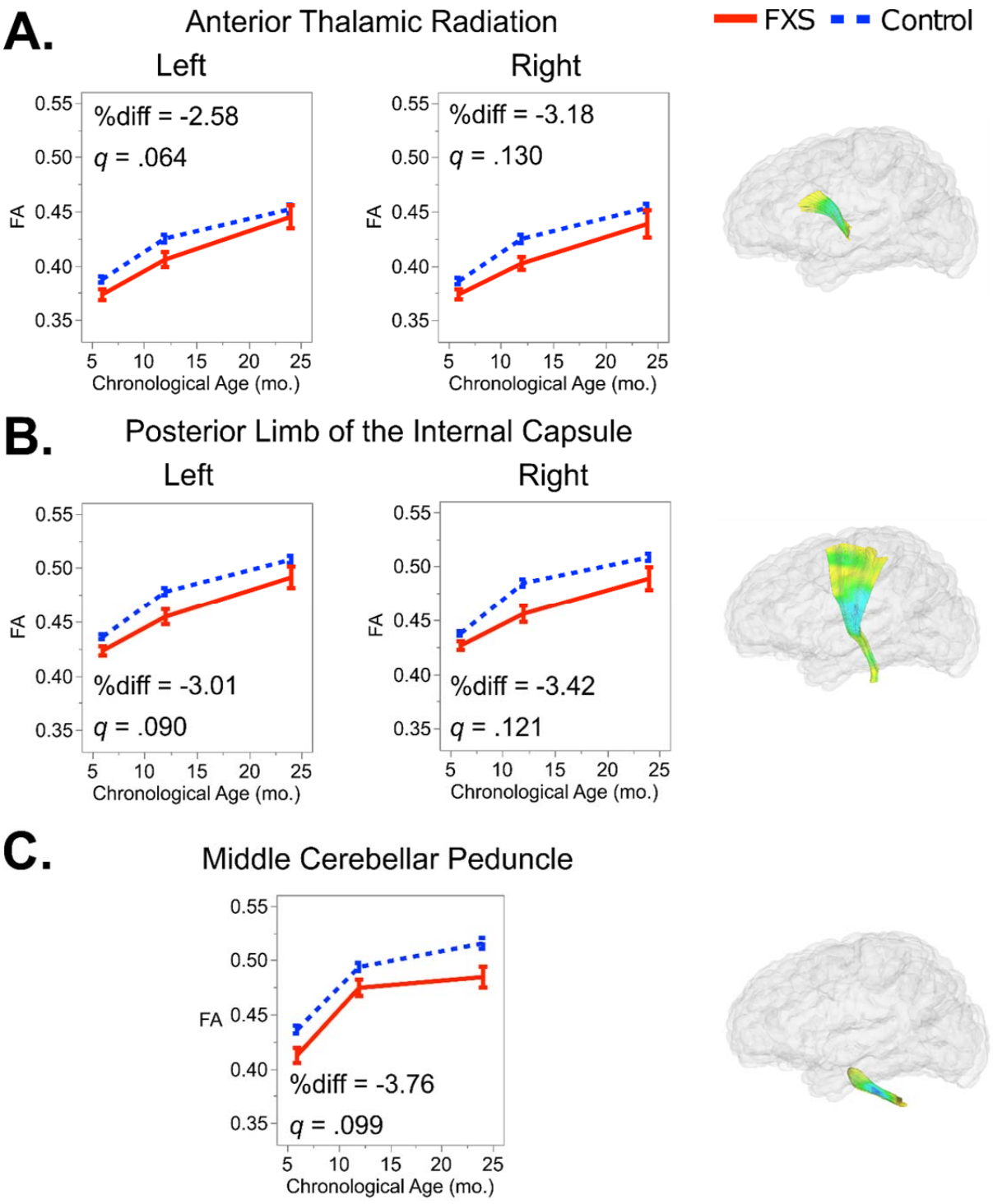
eFigure 1. Infants with Fragile X Syndrome Had Lower FA Than Controls in Four Sections of the Corpus Callosum, Section I (Projections to Prefrontal Regions), Section II (Premotor and Supplementary Motor Cortex), Section III (Projections to the Primary Motor Cortex), and Section Va (Parietal and Temporal Regions)

Error bars = ±1 SEM. q-values are FDR corrected P values for the group main effect. Percent decrease compares least squares means in FA across all time points in FXS compared to controls.



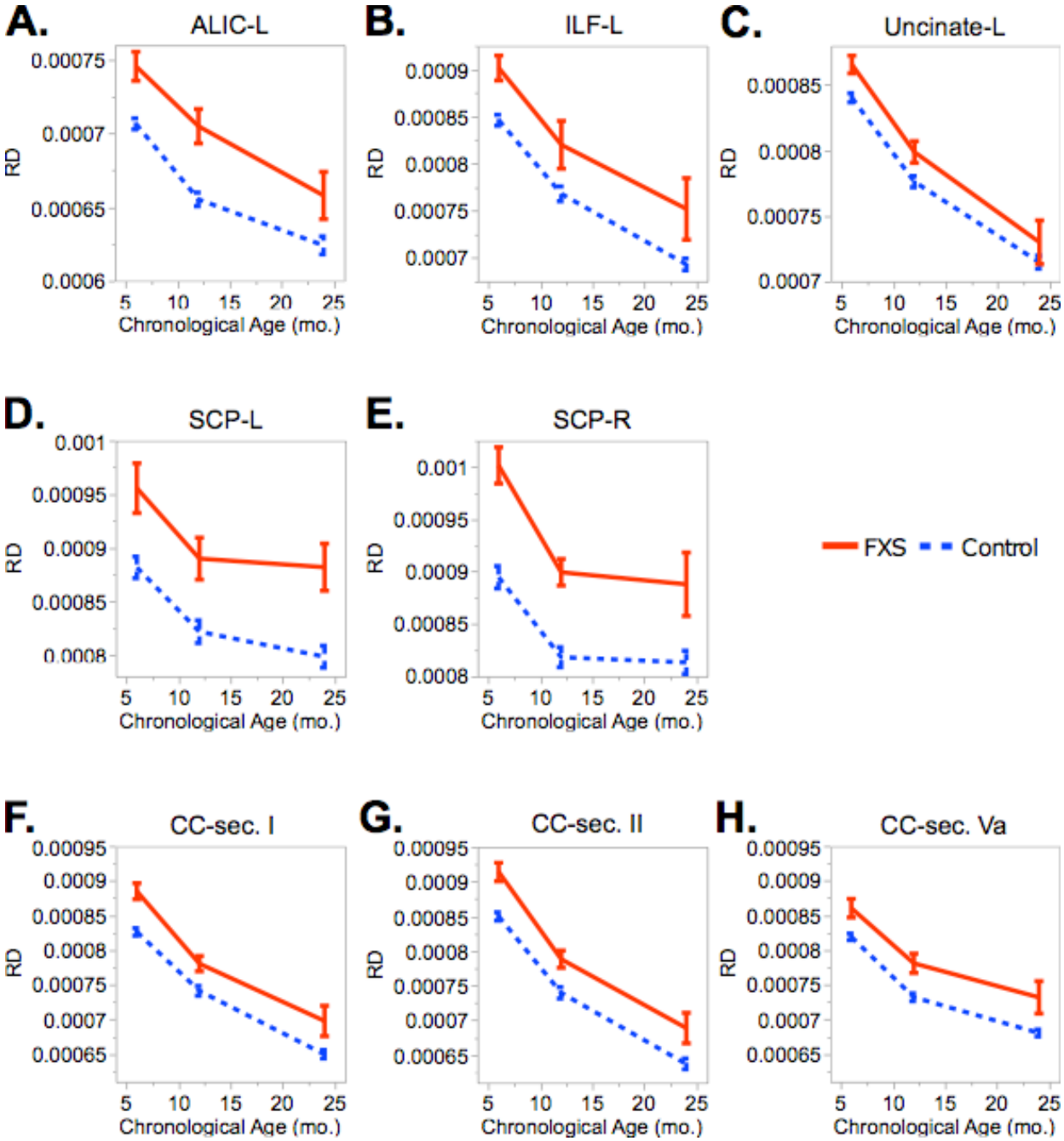
eFigure 2. Infants With Fragile X Syndrome and Control Infants Did Not Differ in FA of Anterior Thalamic Radiations (Panel A), Posterior Limbs of the Internal Capsule (Panel B), Middle Cerebellar Peduncles (Panel C)

Error bars = ±1 SEM. q-values are FDR corrected P values for the group main effect. Percent decrease compares least squares means in FA across all time points in FXS compared to controls.



eFigure 3. Infants With Fragile X Syndrome Had Higher RD Than Controls in the Left Anterior Limb of the Internal Capsule (ALIC-L, Panel A), Left Inferior Longitudinal Fasciculus (ILF-L, Panel B), Left Uncinate Fasciculus (Uncinate-L, Panel C), Left and Right Superior Cerebellar Peduncles (SCP-L, Panel D; SCP-R, Panel E), and Three Sections of the Corpus Callosum, Section I (Panel F), Section II (Panel G), Section Va (Panel H)

Error bars = ±1 SEM.



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