

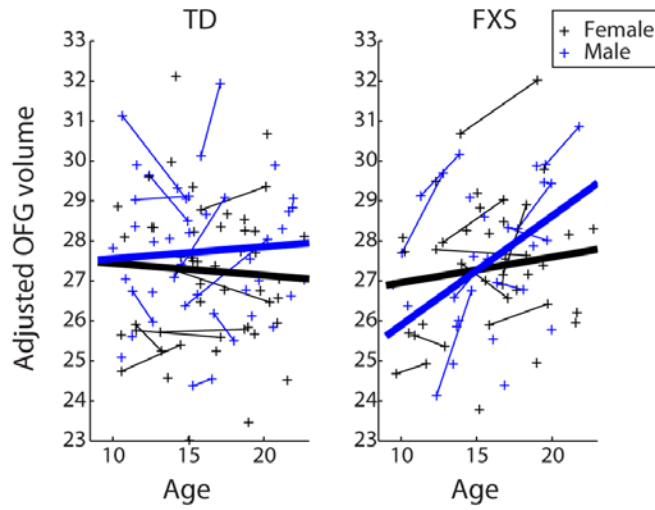
## Supplemental Information

### Supplemental Materials and Methods

#### *FreeSurfer processing*

Cortical reconstruction and volumetric segmentation were performed with the FreeSurfer 4.5 image analysis suite, which is documented and freely available for download online (<http://surfer.nmr.mgh.harvard.edu/>). Briefly, this processing includes removal of non-brain tissue using a hybrid watershed/surface deformation procedure (1), automated Talairach transformation, segmentation of the subcortical white matter and deep gray matter volumetric structures (including hippocampus, amygdala, caudate, putamen, ventricles) (2; 3), intensity normalization (4), tessellation of the gray matter white matter boundary, automated topology correction (5; 6), and surface deformation following intensity gradients to optimally place the gray/white (main) and gray/cerebrospinal fluid (pial) borders at the location where the greatest shift in intensity defines the transition to the other tissue class (7-9). Once the cortical models were complete, regional volumes were extracted by surface inflation (10), registration to a spherical atlas which utilizes individual cortical folding patterns to match cortical geometry across subjects (10), and parcellation of the cerebral cortex into units based on gyral and sulcal structure (3; 11). The main and pial surfaces were visually inspected, and where needed, appropriate manual corrections were performed as per the FreeSurfer Tutorial (<http://surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial>). All raters who performed manual editing of FreeSurfer derived data were trained to achieve inter-rater reliability of  $\geq 0.95$  (intraclass correlation coefficient) with gold-standard datasets for all regions of interest. Volume estimates for middle frontal gyrus were obtained by summing FreeSurfer estimates for rostral and caudal middle frontal gyri, and inferior frontal gyrus by summing pars opercularis, pars triangularis, and pars orbitalis, respectively. Estimates for the orbitofrontal gyrus (OFG) were obtained by summing volumes of lateral and medial

OFG. Amygdala segmentations were found to be less reliable, and were therefore not analyzed in this study.



**Figure S1. Development of orbitofrontal gyrus (OFG) volume.** Bilateral OFG volumes plotted against age, separately for typically developing (TD; left), and Fragile X syndrome (FXS; right), volumes adjusted for total gray matter. Regression lines estimated from linear mixed models are plotted. The age x diagnosis ( $p < 0.05/4$ ) interaction is significant in this model, indicating a significant group difference in regional development.

**Table S1. Medications.** Among the Fragile X syndrome (FXS) participants 17 were taking anti-depressants, 3 antiepileptic medications, 9 stimulants, 3 atypical antipsychotics, and 2 thyroid hormone supplementations.

<b>Diagnostic Group</b>	<b>Medications (# participants and type)</b>
FXS	17 anti-depressants 3 antiepileptic 9 stimulants 3 atypical antipsychotics 2 thyroid hormone supplementations

**Table S2. Raw volume values for ROIs (cm<sup>3</sup>), averaged within age groups.**

Region of interest			9-13 y		13-16 y		16-19 y		19-22 y	
			mean	SD	mean	SD	mean	SD	mean	SD
Total gray matter (cm <sup>3</sup> )	TD	F	558.12	29.12	530.93	32.61	504.9	38.91	504.17	37.01
		M	582.48	46.4	561.39	30.26	545.38	48.58	535.26	50.32
	FXS	F	538.43	48.04	503.61	31.12	505.38	25.57	496.56	40.56
		M	619.26	47.87	566.83	62.55	544.63	23.04	541.21	61.5
Total white matter (cm <sup>3</sup> )	TD	F	453.76	32.01	485.07	33.59	507.85	41.99	485.76	26.99
		M	503.66	44.38	550.89	43.42	563.75	88.31	563.39	74.46
	FXS	F	452.24	47.92	465.2	31.18	492.98	38.02	495.53	40.85
		M	548.54	62.98	585.61	44.7	582.91	51.3	614.3	31.94
Orbitofrontal gyrus (cm <sup>3</sup> )	TD	F	28.57	1.75	26.93	2.54	25.18	2.22	25.6	1.99
		M	29.77	3.52	28.91	1.32	27.92	3.94	27.65	3.01
	FXS	F	26.71	3.66	25.95	2.61	26.05	1.7	25.24	3.22
		M	31.42	3.44	28.67	3.91	27.8	1.92	29.1	2.37
Inferior frontal gyrus (cm <sup>3</sup> )	TD	F	26.86	2.96	26.29	2.32	24.81	2.51	24.36	2.65
		M	29.29	2.84	27.74	2.45	27.42	5.06	26.16	3.89
	FXS	F	25.95	3.1	24.47	2.01	24.5	2.39	22.8	3.26
		M	31.1	1.98	29.38	3.46	26.6	3.75	25.08	3.88
Dorsal frontal (MFG/SFG) (cm <sup>3</sup> )	TD	F	111.38	7.78	105.34	9.67	98.47	11.88	101.34	8.55
		M	115.5	10.94	108.77	6.2	105.85	10.59	103.73	12.97
	FXS	F	104.84	13.14	99.67	10.15	103.88	4.76	95.75	10.11
		M	116.92	13.5	111.15	17.79	110.76	5.46	108.5	16.5
Caudate (cm <sup>3</sup> )	TD	F	7.83	1.22	7.57	0.86	7.76	0.66	7.34	0.81
		M	8.13	0.78	8.12	0.83	7.55	1.58	8.11	0.86
	FXS	F	8.14	0.71	8.32	0.63	8.37	0.82	8.64	0.83
		M	9.12	1.5	9.94	0.91	10.17	1.19	9.94	0.85
Thalamus (cm <sup>3</sup> )	TD	F	14.4	1.17	14.44	0.83	14.73	0.93	14.73	0.77
		M	15.28	1.63	16.27	1.69	16.03	2.03	16.28	1.62
	FXS	F	14.5	1.04	14.75	0.85	14.88	1.1	14.38	1
		M	16.05	1.14	16.43	0.94	16.89	1.17	17.24	1.25
Pallidum/Putamen (cm <sup>3</sup> )	TD	F	15.03	1.06	14.98	0.9	15.01	1.18	14.48	0.73
		M	16.28	1.63	16.42	1.51	15.76	2.56	15.66	1.92
	FXS	F	15.14	0.89	15.09	1.05	15.08	1	14.23	1.24
		M	16.78	1.54	17.29	1.34	16.17	1.19	16.15	1.79
Hippocampus (cm <sup>3</sup> )	TD	F	8.22	0.58	8.26	0.57	8.67	0.82	8.65	0.9
		M	8.62	0.57	8.99	0.62	8.79	0.79	9.13	0.81
	FXS	F	8.17	0.61	8.27	0.79	8.47	0.51	8.02	0.93
		M	9.08	0.64	9.07	0.85	9	1.3	8.94	0.95
Insula (cm <sup>3</sup> )	TD	F	13.92	1.02	13.19	1.22	12.91	1.29	13.22	1.07
		M	14.62	1.08	14.65	0.85	13.94	1.59	13.95	1.45
	FXS	F	12.33	1.99	12.41	0.83	12.66	0.9	12.3	1.08

		M	14.86	1.1	14.21	1.45	13.56	0.93	13.48	1.58
Superior temporal gyrus (cm <sup>3</sup> )	TD	F	27.61	2.42	26.69	3.14	25.07	2.85	25.07	2.53
		M	29.12	2.95	27.75	2.4	27.01	3.93	26.82	3.81
	FXS	F	25.99	3.27	24.59	1.4	24.59	2.2	24.73	2.63
		M	31.28	3.87	28.61	5.74	25.21	1.53	26.67	3.99
Fusiform gyrus (cm <sup>3</sup> )	TD	F	21.42	2.69	20.83	3.26	19.72	1.89	20.58	2.94
		M	23.08	3.2	22.7	2.76	21.56	1.83	21.83	2.74
	FXS	F	22.01	3.03	19.9	2.16	19.02	2.42	19.81	2.5
		M	25	3.02	21.65	2.7	22.54	2.18	19.77	2.7

F, female; FXS, Fragile X syndrome; M, male; MFG, middle frontal gyrus; ROI, region of interest; SFG, superior frontal gyrus; TD, typically developing.

**Table S3. Region of interest models in FXS vs. TD.**

	Parameter	$\beta$ (SE)	$t$ (df)	$p$ -value (uncorrected)
Orbitofrontal gyrus	Dx	-0.04 (0.15)	-.13 (134.2)	$p = 0.9$
	Gender	0.30 (0.30)	1.03 (134.2)	$p = 0.3$
	Dx*Gender	-.26 (0.6)	-.45 (134.2)	$p = 0.66$
	Age	0.09 (0.03)	2.5 (132)	$p = 0.012$
	Dx * Age	0.17 (0.07)	2.5 (132)	$p = 0.014$
	Gender * Age	0.14 (0.07)	2.0 (132)	$p = 0.043$
	Dx * Gender * Age	0.14 (0.13)	1.1 (132)	$p = 0.287$
Frontal Inferior frontal gyrus	Dx	-0.29 (0.41)	-.73 (130)	$p = 0.47$
	Gender	0.76 (0.41)	1.9 (130)	$p = 0.07$
	Dx*Gender	0.26 (0.81)	0.32 (130)	$p = 0.75$
	Age	0.011 (0.04)	0.3 (80.9)	$p = 0.76$
	Dx * Age	-0.097 (0.08)	-1.2 (80.9)	$p = 0.22$
	Gender * Age	0.003 (0.08)	.036 (80.9)	$p = 0.97$
	Dx * Gender * Age	0.19 (0.16)	1.3 (80.9)	$p = 0.22$
Middle / superior frontal gyri	Dx	0.54 (0.93)	.58 (129.7)	$p = 0.561$
	Gender	-1.1 (0.93)	-1.18 (129.7)	$p = 0.24$
	Dx*Gender	0.3 (1.86)	0.163 (129.7)	$p = 0.87$
	Age	0.006 (0.095)	.066 (95)	$p = 0.947$
	Dx * Age	0.45 (0.19)	2.38 (95)	$p = 0.019$
	Gender * Age	0.42 (0.19)	2.22 (95)	$p = 0.028$
	Dx * Gender * Age	1.07 (0.38)	2.79 (95)	$p = 0.006$

Table S3. continued

	Parameter	$\beta$ (SE)	$t$ (df)	$p$ -value (uncorrected)	
Sub- cortical	Dx	1.32 (0.14)	9.0 (136.6)	$p < 0.001$	
	Gender	0.54 (0.14)	3.7 (136.6)	$p < 0.001$	
	Dx*Gender	0.96 (0.29)	3.3 (136.6)	$p = 0.001$	
	Caudate	Age	0.028 (0.014)	2.0 (86.2)	$p = 0.047$
	Dx * Age	0.03 (0.03)	1.1 (86.2)	$p = 0.281$	
	Gender * Age	-0.003 (0.03)	-0.12 (86.2)	$p = 0.9$	
	Dx * Gender * Age	-0.036 (0.06)	-0.64 (86.2)	$p = 0.5$	
	Dx	0.50 (0.17)	2.9 (142.6)	$p = 0.005$	
	Gender	1.1 (0.17)	6.1 (142.6)	$p < 0.001$	
	Dx*Gender	0.53 (0.35)	1.6 (170)	$p = 0.13$	
	Thalamus	Age	0.12 (0.02)	5.7 (170)	$p < 0.001$
	Dx * Age	-0.06 (0.43)	-1.3 (170)	$p = 0.183$	
	Gender * Age	0.10 (0.43)	2.3 (170)	$p = 0.02$	
	Dx * Gender * Age	-0.0004 (0.9)	-0.005 (170)	$p = 0.996$	
	Dx	0.45 (0.21)	2.2 (141.5)	$p = 0.03$	
	Gender	0.83 (0.21)	4.0 (141.5)	$p < 0.001$	
	Dx*Gender	0.47 (0.41)	1.1 (141.5)	$p = 0.26$	
	Putamen / Pallidum	Age	0.018 (0.02)	0.8 (136)	$p = 0.43$
	Dx * Age	-0.004 (0.45)	-0.083 (136)	$p = 0.93$	
	Gender * Age	0.006 (0.05)	0.12 (136)	$p = 0.9$	
	Dx * Gender * Age	-0.065 (0.09)	-0.71 (136)	$p = 0.48$	
Dx	0.005 (0.11)	0.041 (132.9)	$p = 0.97$		
Gender	0.31 (0.11)	2.8 (132.9)	$p = 0.007$		
Dx*Gender	0.19 (0.23)	0.82 (132.9)	$p = 0.42$		
Hippocampus	Age	0.06 (0.014)	4.2 (158.3)	$p < 0.001$	
Dx * Age	-0.05 (0.03)	-1.8 (158.3)	$p = 0.07$		
Gender * Age	-0.004 (0.03)	-1.16 (158.3)	$p = 0.87$		
Dx * Gender * Age	-0.004 (0.06)	-0.07 (158.3)	$p = 0.95$		

Table S3. continued

		Parameter	$\beta$ (SE)	$t$ (df)	$p$ -value (uncorrected)
	Insula	Dx	-0.55 (0.12)	-4.5 (135.9)	$p < 0.001$
		Gender	0.32 (0.12)	2.6 (135.9)	$p = 0.01$
		Dx*Gender	0.11 (0.25)	0.44 (135.9)	$p = 0.66$
		Age	0.06 (0.01)	4.9 (110.6)	$p < 0.001$
		Dx * Age	0.003 (0.026)	0.10 (110.6)	$p = 0.92$
		Gender * Age	-0.003 (0.026)	-0.127 (110.6)	$p = 0.9$
		Dx * Gender * Age	0.03 (0.052)	0.58 (110.6)	$p = 0.56$
Temporal- occipital	Superior- Temporal	Dx	-0.40 (0.34)	-1.2 (131.2)	$p = 0.25$
		Gender	-0.19 (0.34)	-0.54 (131.2)	$p = 0.59$
		Dx*Gender	0.50 (0.69)	0.73 (131.2)	$p = 0.47$
		Age	0.11 (0.036)	2.9 (107.3)	$p = 0.005$
		Dx * Age	0.07 (0.07)	0.95 (107.3)	$p = 0.35$
		Gender * Age	0.004 (0.073)	0.06 (107.3)	$p = 0.95$
		Dx * Gender * Age	-0.03 (0.15)	-0.19 (107.3)	$p = 0.85$
	Fusiform	Dx	0.008 (0.35)	0.023 (141.2)	$p = 0.98$
		Gender	0.07 (0.35)	0.19 (141.2)	$p = 0.85$
		Dx*Gender	-0.42 (0.70)	-0.6 (141.2)	$p = 0.55$
		Age	0.065 (0.042)	1.55 (157.3)	$p = 0.12$
		Dx * Age	-0.06 (0.08)	-0.71 (157.3)	$p = 0.48$
		Gender * Age	-0.026 (0.08)	-0.31 (157.3)	$p = 0.76$
		Dx * Gender * Age	0.067 (0.17)	0.40 (157.3)	$p = 0.69$

Dx, diagnosis; FXS, Fragile X syndrome; TD, typically developing.



**Table S4. Behavioral data summary**

<b>Cognitive measure</b>	<b>Diagnosis</b>	<b># of time 1</b>	<b># of time 2</b>	<b># of time 3</b>	<b># of time 4</b>
Spatial relations	TD	76	21	1	
	FXS-female	35	26	8	1
	FXS-male	24	19	9	2
Verbal fluency	TD	79	22		
	FXS-female	40	25	19	1
	FXS-male	24	19	11	2

FXS, Fragile X syndrome; TD, typically developing.

### Supplemental References

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