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Trajectories of Early Brain Volume Development in Fragile X and Autism RH: Trajectory of Brain Volume in Fragile X

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Abstract

Objective—To examine patterns of early brain growth in young children with fragile X syndrome (FXS) compared to a comparison group (controls) and a group with idiopathic autism.

Method—The study included 53 boys between 18–42 months of age with FXS, 68 boys with idiopathic autism (ASD), and a comparison group of 50 typically-developing and developmentally-delayed controls. We examined structural brain volumes using magnetic resonance imaging (MRI) across two timepoints between ages 2–3 and 4–5 years and examined total brain volumes and regional (lobar) tissue volumes. Additionally, we studied a selected group of subcortical structures implicated in the behavioral features of FXS (e.g., basal ganglia, hippocampus, amygdala).

Results—Children with FXS had greater global brain volumes compared to controls, but were not different than children with idiopathic autism, and the rate of brain growth between ages 2 and 5 paralleled that seen in controls. In contrast to the children with idiopathic autism who had generalized cortical lobe enlargement, the children with FXS showed a specific enlargement in temporal lobe white matter, cerebellar gray matter, and caudate nucleus, but significantly smaller amygdala.

Conclusions—This structural longitudinal MRI study of preschoolers with FXS observed generalized brain overgrowth in FXS compared to controls, evident at age 2 and maintained across ages 4–5. We also find different patterns of brain growth that distinguishes boys with FXS from children with idiopathic autism.

Keywords

fragile X syndrome; autism; children; brain MRI; brain volume

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Introduction

Fragile X syndrome (FXS) is a well characterized X-linked genetic disorder and the leading heritable cause of intellectual disability.[1] Boys with the FXS full mutation have been reported to show social deficits with peers, abnormalities in communication, unusual responses to sensory stimuli, stereotypic behavior, social avoidance, and gaze aversion.[2–7] Approximately one third of individuals with FXS meet criteria for autistic disorder,[3,8] while 1–3% of individuals with autistic disorder are identified to have FXS. Autism is a clinically heterogeneous disorder without a known specific genetic mechanism. Conversely, FXS is a single gene disorder and provides a more circumscribed way to examine the impact of aberrant brain mechanisms and development.

Giedd et al.[9] have demonstrated the utility of longitudinal brain development studies in typical development and documented nonlinear brain growth. This approach is particularly relevant when examining brain changes in a neurodevelopmental disorder that many involve nonlinear changes over time. There is a critical need for longitudinal brain imaging studies in conditions such as fragile X, where behavioral changes across development have been reported (e.g., increase in repetitive behaviors at school-age). Better characterization of the changing patterns in brain growth over time may provide clues to underlying mechanisms and can better depict the phenotype of FXS across development.

This report is from a longitudinal magnetic resonance imaging (MRI) study of brain development in preschoolers with FXS. In a cross-sectional volumetric MRI study of this sample,[10] toddlers with FXS (18–42 months old) were found to have enlarged caudate nucleus (CN) and smaller amygdala (AMY) volume compared to controls, while the toddlers with idiopathic autism (iAUT) had a different profile, showing only modest CN enlargement and significantly larger AMY. Voxel based morphometry (VBM) studies of this sample examined FXS compared to controls and found larger gray matter volume (GMV) in CN, thalamus, and fusiform gyrus and decreased GMV in cerebellar vermis present at both timepoints in the FXS group.[11,12] White matter volume (WMV) was observed to be larger in frontal striatal regions in the boys with FXS compared to controls and became more pronounced over time.[12] A cross-sectional comparison study using VBM[13] examined the FXS group compared to iAUT and observed different patterns of brain volumes in the iAUT and control group compared to FXS. Notably, using this different methodology the pattern of greater CN and reduced AMY that was seen in the volumetric ROI analysis[10] was seen in the FXS group versus iAUT. These VBM studies provide converging data for our structural MRI findings pointing to a specific pattern of larger CN and implicate cerebellar, thalamic, and temporal lobe pathways associated with FXS.

In this paper, we extend our previous work (10) by comparing the longitudinal brain volume data from this sample of boys with FXS to a sample of boys with iAUT. We addressed three questions about our group FXS: (1) Were there differences in brain volumes compared to age and IQ matched male controls? (2) Were there differences in brain volumes compared to males with iAUT? and (3) What was the pattern of brain volumes in those boys with FXS who were comorbid for autism? For each of these questions, we examined global brain volumes (e.g., GMV, WMV), regional brain volumes (e.g., cortical lobes), and a set of selected substructures of interest (e.g., CN, AMY).

Method

Sample

The study included 53 male children with fragile X syndrome (FXS), 68 children with idiopathic autism (iAUT), and 50 comparison cases who were enrolled in this longitudinal study between 18–42 months of age and received an initial behavioral assessment and brain MRI scan. Approximately two years later, at age 4-5 years old, this cohort of children received a repeat assessment and MRI. At follow-up, the sample contained 39 boys with FXS, 44 boys with iAUT, and 26 comparison cases. The comparison group was comprised of typically developing children (TYP) and children with developmental delay (DD) who had no evidence of a pervasive developmental disorder (PDD). The group with ASD was observed to be lower functioning (estimated IQ in the 50s) while the TYP fell in the average range (estimated IQ ~100). The DD control group was included to enrich the comparison sample for low IQ non-autistic subjects since our sample of FXS and iAUT was comprised of children with lower intellectual functioning.

See Table 1 for a description of subject characteristics. At time 1 there were a total of 50 children in the comparison group (31 TYP, 19 DD); at time 2 there were 26 cases (19 TYP, 7 DD). For exploratory analyses, a subgroup of the FXS sample was identified who met criteria for autism on the Autism Diagnostic Interview–Revised (ADI-R) and ADOS (FXS +Aut) (time 1 = 16, time 2 = 13), and the remaining subjects were identified as not meeting criteria (FXS-Aut) (time 1 = 37, time 2 = 26). All subjects in the study were male.

A full description of the ascertainment and inclusion criteria is reported elsewhere,[10] but a brief summary is included here. All subjects were enrolled between 18–42 months of age (time point 1) and seen for a repeat assessment approximately 24 months after their initial assessment (time point 2). At study enrollment, medical records and developmental history were reviewed and records were re-evaluated at time point 2. Inclusion in the FXS group required DNA testing confirming the fragile X full mutation. Children with iAUT were referred after receiving a clinical diagnosis of autistic disorder which was confirmed with clinical testing (see below). Subjects with DD were referred only if they had no known identifiable cause for their delay (e.g., prematurity, genetic or neurological disorder) and had no indication of a pervasive developmental delay. The TYP subjects were recruited from the community and were screened for an autism spectrum disorder (ASD). All subjects with iAUT, DD, and TYP were excluded for evidence of fragile X syndrome, Tuberous Sclerosis (TS), gross CNS injury (e.g., cerebral palsy, significant pregnancy complications or perinatal/postnatal trauma, drug exposure), prematurity (<34 weeks), low birth weight (<2000 g), seizures, and significant motor or sensory impairments. Additionally, the DD and TYP children were screened for autism with the Childhood Autism Rating Scale[14] and excluded if they met the cutoff for autism (> 25 total score). All autistic and DD subjects received DNA testing to exclude FXS.

Study approval was acquired from the University of North Carolina (UNC) and Stanford Institutional Review Boards and written informed consent was obtained by getting parental (or custodial guardian) consent for each subject.

Clinical Assessment

Medical records and developmental history were reviewed for all subjects at the time of entry to the study. Inclusion in the FXS group required DNA testing confirming the fragile X full mutation as diagnosed with standard Southern Blot technique. These children also received testing for the fragile X mental retardation protein (FMRP) expression by calculating the percentage of peripheral lymphocytes containing FMRP using immunostaining techniques.[15] Some subjects with autism were included from another study

and criteria for that study have been described elsewhere.[16] Briefly, children were referred to that study after receiving a clinical diagnosis of autism, which was then confirmed by our team using the ADI-R[17] and the Autism Diagnostic Observation Schedule-G (ADOS-G)[18] scores. Subjects were only included in the iAUT group if they met criteria for autism in all domains of the ADI-R and obtained ADOS-G consistent with autism. The same assessments were used at time point 2 (age 4–5 years) and a small subset of subjects failed to meet the original study criteria for autistic disorder (e.g., ADI-R, ADOS-G, *DSM-IV*) but continued to show evidence of symptoms consistent with a PDD-NOS diagnosis. These subjects were classified as PDD. The iAUT sample therefore included 61 autism and 7 PDD subjects at time 1, and 39 autism and 5 PDD subjects at time 2. Inclusion in the DD group was defined as having significant global delays (developmental IQ < 80), scores consistent with DD on the other assessment measures, no known identifiable cause for their delay (on medical record review), and no indication of PDD. Inclusion in the TYP group was defined as having average developmental and cognitive abilities (i.e. developmental IQ > 85).

All subjects were given a battery of measures including the Mullen Scales of Early Learning,[19] Differential Abilities Scale (DAS)[20] (at time 2 only), the Vineland Adaptive Behavior Scales,[21] behavioral rating scales (e.g., Aberrant Behavior Checklist,[22] Repetitive Behavior Scales[23]), and a standardized neurodevelopmental examination to exclude subjects with any notable dysmorphology, evidence of neurocutaneous abnormalities, or other significant neurological abnormalities.

Table 2 presents the cognitive and adaptive functioning characteristics of the sample. Many of the subjects with FXS, iAUT, and DD failed to obtain a valid standard score on the DAS at time point 2, so we only provide estimates of cognitive functioning from their Mullen.

MRI Acquisition

All subjects were scanned on a 1.5 Tesla GE Signa MRI scanner (GE Imagine Systems, Milwaukee, WI) at either the Lucile Packard Children's Hospital (Stanford) or the Duke–UNC Brain Imaging and Analysis Center (BIAC). Image acquisition was designed to maximize gray/white tissue contrast for the pediatric brain and included: (1) a coronal T1 IR Prepared: T1 300 msec, TR 12 msec, TE 5 msec, 20° flip angle, at 1.5 mm thickness with 1 NEX, 20 cm FOV; and 256 x 192 matrix; (2) a coronal PD/T2 2D dual FSE, TR 7200 msec, TE 17/75 msec, at 3.0 mm thickness with 1 NEX, 20 cm FOV, and 256 x 160 matrix. A series of localizer scans and a set of phantoms was used to standardize assessments across sites and time (for the longitudinal study). The in-plane voxel resolution for the T1 and PDT2 scans are as follows: T1 scans: 0.78125 x 0.78125 x 1.50; PD/T2 scans: 0.78125 x 0.78125 x 3.0. Both scans are then resampled to isotropic (1.01562 x 1.01562 x 1.01562) with our BRAINS2 software during the registration process.

Subjects with FXS, autism and DD were scanned using sedation administered by a sedation nurse and/or under the supervision of a pediatric anesthesiologist. Physiological monitoring was conducted throughout the scan and recovery. TYP subjects were scanned without sedation, in the evening, while sleeping. At age two all the TYP subjects were scanned in the evening, while sleeping. At age 4, some of the TYP subjects (n=5) were scanned while awake, after completing a behavioral training protocol to learn to lie still in the scanner. The remaining TYP subjects were scanned while sleeping for time point 2. All MRI scans were reviewed by a pediatric neuroradiologist and screened for significant abnormalities (e.g., malformations, lesions, etc.).

Image Processing

The image processing procedures for this data are identical to those described in the initial volumetric paper from this longitudinal study.[10] The primary components are briefly reported here for reference. Scans first underwent quality control checks to determine if they were of sufficient quality to process. The T1 and PD/T2 scans were then registered and aligned into a standardized plane along an AC-PC (anterior-posterior commissure) axis.[24] The co-registered and aligned images were then processed for tissue segmentation using the Expectation Maximization Segmentation (EMS) pipeline.[25] An "averaged" pediatric probabilistic brain atlas was aligned to each subject brain using a linear, affine transformation in a fully automated procedure. The automated procedure included bias estimation, inhomogeneity correction, and non-brain stripping procedures. Gray, white, and CSF tissue segmentations were produced for each subject. Total brain volume (TBV) measures included total gray and white matter and all CSF. Total tissue volume (TTV) included all gray and white matter in the cerebrum (cerebral cortical volume), cerebellum, and brainstem.

Regional lobe measurements were obtained using a manually parcellated template (atlas) MRI of pediatric brain developed by our group (16), which was then mapped onto each subject brain using a fluid high-dimensional deformation algorithm (described in Hazlett et al., 2005).[16] Delineated regions included the frontal, temporal, parietal, and occipital lobes, cerebellum, corpus callosum, and a "subcortical area" (basal ganglia, thalamus, deep white matter, and brainstem). The insula and cingulate gyrus were also defined, but for the purposes of these analyses the insula was included in the cerebral cortex measure and cingulate with the frontal/parietal lobes. Cortical label maps were combined with the EMS tissue classified images to produce gray/white/CSF volumes for each of these lobe compartments.

Selected subcortical structures were measured using region-of-interest protocols which are described elsewhere.[10] Right and left volumes for the caudate nucleus (CN), putamen (PUT), globus pallidus (GP), amygdala (AMY), and hippocampus (HIPP) were obtained using semi-automated or manual protocols.

Data Analysis

Descriptive statistics and data plots were first examined to look for outliers. No anomalous data was observed or removed. A priori hypotheses were tested using general linear mixed models with repeated measures in SAS 9.1. In all models brain volume was the dependent variable and diagnostic group (FXS, iAUT, DD, TYP), age, and IQ were independent variables. Time was entered as a repeated measure. Group differences in developmental patterns were examined by testing the age X group interactions. For each group of analyses (total brain, cortical lobes, substructures) two models were fit to the data: (*1*) the first model included group, age and IQ, (*2*) the second model added TBV as a covariate to evaluate whether any of the differences in brain volumes were disproportionate to differences observed for TBV (in the lobes or subcortical regions). All analyses adjusted for the effects of age and IQ ratio on the measured brain volume by including them as covariates. Data collection site (UNC, SU) was not included as a predictor because no systematic difference in brain volumes (GM, WM, CSF, TTV, and 5 substructures of interest) were observed between sites.[12] Laterality was assessed by examining the significance of interactions between group and hemisphere (left or right 'side').

Age and IQ were scaled to aid interpretation of the results. Age was centered at 3.5 years which was close to the overall mean of 3.6 years. An IQ ratio was calculated by dividing the child's age equivalent score on the Mullen Visual Reception subscale by the child's actual

age. This allows a more precise measure of children's nonverbal abilities who would otherwise score at the lower end of the standardized scale and be assigned values below a basal standard score ('<49'). This IQ ratio was then centered which was the mean for all observations. The rescaling of these variables results in all main effects being estimated at these values unless otherwise specified.

Analyses were organized to examine the following group comparisons: (1) FXS compared to controls, (2) FXS compared to iAUT, and (3) FXS subgroups (with or without autism) compared to iAUT. Our first model focused on three group comparisons (FXS vs. iAUT vs. Controls). For these comparisons, combined estimates for 'controls' (DD + TYP) was created using post-estimation commands to create weighted averages. By using a weighted average of the subgroups the combined group estimates are accurate estimates of the means, while the possible error variance that could be accounted for by mean group differences is minimized. Our secondary analysis included the subgroup comparisons (FXS+Aut, iAUT, DD, TYP). However, only a single model was fit to obtain these estimates.

Tables 1 and 2 describe the sample characteristics. Age differences were observed (the TYP subgroup was slightly younger than the other groups) and, therefore, age was included as a covariate. Developmentally-delayed subjects were included in the control group to control for IQ differences. While IQ was not found to be a significant predictor between groups, comparisons were run with IQ as a covariate to be conservative. Study retention rates were as follows: FXS+Aut (81%), FXS-A (70%), TYP (61%), iAUT (63%), and DD (37%). We saw no differences in our results when we removed subjects who did not complete both time points for the study, so the results below include those cases. We also examined descriptive statistics for the FXS and then comparison group and did not find any significant difference in age, developmental IQ, adaptive functioning, or on symptom severity as reported on the ADI-R (for the FXS and iAUT groups only) between subjects who completed the study (2 time points) versus those who dropped out (1 time point).

Differences between the groups controlling for age and IQ were examined. While all the groups showed an increase over time in brain volume for all areas measured, there were no significant group differences in the rate of brain growth over time. Because age by group interactions were not significant, only the main effect of group (averaged over time) is reported. Interactions with side (right/left) were not significant, therefore results are reported as total volume (sides combined).

Results

(1) FXS compared to controls

Raw means for FXS and control groups for total brain (TBV), total gray matter (TGM) and total white matter (TWM) volumes are displayed in Table 3.

As age by group interactions were not significant, only main effect of group (averaged over the 2 to 4–5 year age interval) is reported. Mean group differences are reported in Table 4. Subjects with FXS had significant enlargement in TBV, total tissue volume (TGM+TWM, or TTV), TGM, and TWM compared to controls after adjusting for age and IQ. The regional comparisons (cerebrum, cerebellum, cortical lobes) were adjusted for age, IQ and TBV. Temporal lobe white matter volumes remained significantly increased in the FXS group compared to controls, while small but significant reductions in cerebral gray matter, cerebellar white matter, and frontal gray matter were observed. Exploratory examination of the control subgroups (DD, TYP) revealed that total brain enlargement in FXS compared to controls was largely driven by smaller brain volumes in the DD group with the exception of temporal lobe white matter volume, which was significantly enlarged in the FXS group

compared to both the DD ($p<.001$, 10% difference) and TYP ($p<.001$, 10% difference) controls after adjusting for age, IQ, and TBV.

Using data adjusted for age only, the FXS group had significant enlargement in caudate (40%), globus pallidus (18%), and putamen (8%). This was true for total (combined) volume as well as for right/left side. After controlling for age, IQ, and TBV, the findings remained significant although the percent of enlargement observed was slightly less: caudate (34%), globus pallidus (15%); and putamen (5%). The robust enlargement in the caudate and globus pallidus remained significant compared to both DD and TYP subgroups (using either volumes adjusted only for age, or for age/IQ/TBV). The enlargement in the putamen was significant for both subgroups (DD and TYP) when examining age only corrected data, but only for the TYP subgroup when using all covariates (age/IQ/TBV). The total, left, and right amygdala volumes were smaller in the FXS group compared to controls (−9%). Amygdala volumes (total, left, and right) were smaller compared to all subgroups (by about 4–6%), but differences were significant with the DD subgroup only when adjusting for age/IQ/TBV (by 11%). Left hippocampus was also significantly smaller in FXS compared to controls (−7%) and the TYP subgroup (−7%) when controlling for age/IQ/TBV. Comparisons for the substructure volumes, controlling for age/IQ/TBV, are displayed in Table 5.

There was no difference between the FXS and controls in their rate of brain growth during the age interval studied (2–5 years) for TBV, total tissue volume (TGM+TWM, or TTV), TGM, TWM, and total CSF. These results indicate that differences in overall volume, first observed at age 2, are maintained throughout the age interval i.e., the rate of change over the interval is no different between cases and controls. The trajectories of brain growth for TBV are displayed in Figure 1. The pattern for substructures (CN, AMYG, etc.) did not reveal any group differences.

(2) FXS compared to iAUT

There were no significant brain volume differences in global volumes (TBV, TTV, TGM, TWM) between individuals with FXS and iAUT. We have previously demonstrated increased volume of these structures in individuals with iAUT versus controls (with typical development and developmental-delay, who did not have either autism or FXS) (16). After controlling for age, IQ, and TBV, the FXS group had significantly enlarged total cerebellum (diff 5.09 (1.7), $p = .003$) and cerebellar gray matter (diff 4.97 (1.5), $p=.001$). Total cerebral cortical volume was significantly less in FXS compared to iAUT (diff −11.02 (3.3), $p<.001$), due to smaller cortical gray matter volume in FXS (diff −11.45 (2.8), $p<.001$). The FXS group had significantly greater temporal lobe WM volume (diff 3.25 (0.7), $p<.001$) but all frontal lobe volumes were significantly smaller in FXS compared to the iAUT group (frontal lobe diff −10.82 (3.1), $p<.001$; frontal GM diff −7.67 (2.3), $p<.001$; frontal WM diff −3.15 (1.3), $p=.013$) (Figure 2). There were no significant differences observed in any of the parietal- occipital volumes measured (total, GM, WM). The FXS group had significantly greater caudate volume (29%), globus pallidus (7%), and putamen (6%) versus iAUT, and findings did not differ with age-only adjusted volumes or the age/IQ/TBV adjusted. Both amygdala and hippocampus were significantly smaller in the FXS group compared to the iAUT group, using either age only or age/IQ/TBV adjustments.

There was no difference between the FXS and iAUT in their rate of brain growth during the age interval studied (2–5 years) for any of the brain volumes we examined.

(3) FXS subgroups (with or without autism) compared to iAUT Table 6 provides scores for individuals with FXS who met criteria for autism

(FXS+Aut) and those with FXS who did not meet criteria for autism (FXS-Aut) on the autism behavioral assessment measures (i.e., ADI-R and ADOS). Comparisons between FXS+Aut subgroup and the iAUT group revealed no differences in total brain volumes (TBV, TGM, TWM), but significantly larger total cerebellum (p=.03, 4%) and cerebellar GM (p=.01, 5%), and significantly smaller total cerebral cortex (p=.006, -1%) and cerebral cortical GM (p<.001, −2%) observed in the FXS+Aut subgroup versus iAUT. The FXS+Aut subgroup also had significantly larger temporal lobe WM (p=.001, 8%) and smaller frontal lobe volumes (total p=.004, −3%; WM p=.04, −3%; GM p=.007, - 3%) compared to the iAUT group.

The FXS-Aut subgroup had significantly larger total cerebellum (p=.001, 5%) and cerebellar GM (p<.001, 5%) volumes, and significantly less total cerebral cortex (p=.002, −1%) and cerebral cortex GM (p=.003, −1%) versus iAUT and significantly larger temporal lobe WM (p<.001, 8%) and reduced frontal lobe volumes (total p<.001, −3%; WM p=.02, −2%; GM p<.001, −3%) versus the iAUT group.

There were no differences between the FXS subgroups (FXS+Aut vs. FXS-Aut) for any of the subcortical volumes, although the trajectory between 2 and 4-5 years of age did appear to be different, but not significantly (see Figure 3). The pattern of enlarged caudate, globus pallidus, and putamen, and smaller amygdala and hippocampus volumes was the same for the FXS with and without autism subgroups compared to iAUT (see Table 7).

Discussion

Children with FXS were observed to have increased total brain, total tissue, total gray matter, total white matter, and temporal lobe white matter in comparison to controls. Cerebral cortex gray matter, cerebellar white matter, and frontal lobe gray matter volumes were noted to be slightly decreased in the FXS group compared to controls. We did not observe any group differences in the rate of brain growth between groups, meaning that the rate of brain growth across the interval from 2 to 5 years of age in children with FXS paralleled that seen in controls. This would suggest that, for the brain regions assessed with the methods described here, brain overgrowth had its onset prior to the first measurement at 2 years of age.

In comparison to a control group composed of both TYP and DD, and a contrast group consisting of children with iAUT, the children with FXS showed a unique pattern of brain volume measurements. Whereas, for the most part, children with FXS showed generalized enlargement of both gray and white matter volume in the cerebral cortical lobes in contrast to controls, enlargement was less robust than that observed in children with iAUT, with the exception of a more striking, disproportionate enlargement of temporal lobe white matter in children with FXS (as compared to both controls and those with iAUT). Temporal lobe white matter volume was the only cortical volume in individuals with FXS to show significant enlargement after adjustment for TBV, suggesting a specific neuroanatomical signature that goes beyond the generalized cerebral cortical volume enlargement seen in both FXS and iAUT. Temporal lobe white matter was also a region observed to be enlarged in the VBM study by Hoeft et al., (2011) (13). Specific differences were also observed in the cerebellum where FXS subjects showed significant enlargement of cerebellar gray matter volume in contrast to individuals with iAUT.

The pattern of subcortical volume changes also showed a specific pattern over time, consistent with previous reports by our group looking at brain volumes at age two years[10]

and voxel-based morphometric measurements throughout this early age interval.[11,12] Specifically, here we report a stable increase in volume, maintained over this two year age interval, in basal ganglia structures, with a striking, persistent increase in caudate volume (34%), followed by globus pallidus (15%) and putamen (5%); along with a decreased volume of the amygdala, predominantly noted on the right side. In contrast to children with iAUT, where we have previously reported enlargement of the amygdala with a more modest but significant enlargement of caudate volume, children with FXS show a pattern of markedly increased caudate volume with significantly decreased volume of the amygdala that is present at 2 and maintained across early development. The robust caudate enlargement (by over 30%) replicates our earlier reports on this sample at age 2–3[10,13] and is consistent with observations in older individuals with FXS.[26,27]

Our findings of a specific pattern unique to FXS (in contrast to iAUT) are consistent with behavioral reports of distinct social and communicative profiles for children with FXS and ASD.[28] Work examining 9–12 month old infants with FXS finds evidence for a specific pattern of motor-sensory deficits that can be distinguished from infants with DD and ASD (by diagnosis at later age).[29] Specifically, problems with motor planning, repetitive movements and repetitive use of objects were observed, making these motor-sensory deficits some of the earliest detectable features of FXS. Our observation of significant basal ganglia enlargement is consistent with these behavioral findings and suggests these brain differences may be present from a very early age, if not prenatally.

Of note, reports about the molecular biology of FXS suggest a possible mechanism for brain growth patterns observed in this study. Harlow et al.[30] has demonstrated that FMRP inhibits the generation of progenitor neurons from radial glia in mouse cerebral cortex, suggesting that lack of FMRP, as seen in FXS, might result in an increased proliferation of progenitor cells and subsequent cerebral cortical overgrowth. Alternatively, FMRP has been shown to act as a regulator of dendritic mRNA.[31] There are a number of hypotheses about the role of FMRP in brain growth that could help explain specific pattern of brain development we find in FXS. Work with the *Fmr1* knock-out mouse has identified FMRP as playing a specific role in neocortical development.[32] FMRP has been shown to act as a regulator for dendritic mRNA[33] and the lack of FMRP has been linked to defects in the differentiation and migration of neural progenitor cells in the neocortex.[33] BDNF and trkB signaling in the neural progenitor cells plays a critical role in normal cortical development, and these signaling pathways function aberrantly in the neuroplast of the FXS knockout mouse.[34] Grossman and colleagues[35] proposed that FMRP acts as a general regulatory process over dendritic spine loss, maturation, and formation, and that this could explain why different brain regions may display different ‘phenotypes’. Our findings for specific patterns of enlargement (e.g., basal ganglia, cerebellum) as well as diminished size (amygdala) in young children with FXS would support this hypothesis.

These findings have significance for expanding our understanding about the neurodevelopmental mechanisms underlying FXS. The presence of early brain differences (evident by age 2) in young children with FXS points to aberrant early brain development in this condition. Examining brain growth in infants with FXS, and perhaps including both full and premutation cases may further illuminate the pathogenesis and provide additional clues for mechanisms to target for intervention during vulnerable periods of brain growth. This study also highlights the importance of longitudinal studies to help define the trajectory of brain development. Studying the trajectory of brain development as children enter school age would provide clues to better understand the effectiveness of targeted pharmacological interventions (e.g., mGluR5) on brain growth. Lastly, these data continue to point to a specific neuroanatomical signature for FXS that differs from that seen in iAUT, suggesting the importance of studying the neurobiological basis of autistic behavior in more

etiologically-defined and etiologically-homogeneous disorders such as FXS, and support the notion that the field may be underestimating the neurobiological heterogeneity underlying autistic behavior.

One limitation of our study is that the brain volume focused on regions-of-interest defined by sulcal-gyral anatomy (e.g., cerebral lobes) or identifiable subcortical boundaries (e.g., hippocampus, caudate). This volumetric approach is complementary to, yet different from a voxel-level morphological approach for elucidating fine-grained neuroanatomical variations, which was used to analyze this FXS cohort in an earlier study from our group.[11–13] In this previous study we detected FXS specific trajectories in various parts of the cerebral cortex, the thalamus and the basal forebrain. Another, limitation is the size of our subgroups of the controls (DD and TYP) was also modest and is a limitation, although the longitudinal design allows for increased power to detect significant differences, particularly with the effect sizes we observed in the basal ganglia. We only examined male children with the full mutation, and therefore our findings may not generalize to females or individuals with the premutation. Lastly, the measures we employed for the behavioral assessment (e.g., ADI-R, ADOS) were developed for categorical diagnosis of autistic disorder and we were therefore limited in the ability to look at dimensional qualities across time.

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References

1. Restifo LL. Mental retardation genes in drosophila: New approaches to understanding and treating developmental brain disorders. Ment Retard Dev Disabil Res Rev. 2005; 11:286–294. [PubMed: 16240406]
2. Baumgardner TL, Reiss AL, Freund LS, Abrams MT. Specification of the neurobehavioral phenotype in males with fragile X syndrome. Pediatrics. 1995; 95:744–752. (1995). [PubMed: 7724315]
3. Bailey DB Jr. Mesibov GB, Hatton DD, Clark RD, Roberts JE, Mayhew L. Autistic behavior in young boys with fragile X syndrome. J Autism Dev Disord. 1998; 28:499–508. [PubMed: 9932236]
4. Bailey DB Jr. Hatton DD, Mesibov G, Ament N, Skinner M. Early development, temperament, and functional impairment in autism and fragile X syndrome. J Autism Dev Disord. 2000; 30:49–59. (2000). [PubMed: 10819120]
5. Bregman JD, Leckman JF, Ort SI. Fragile X syndrome: genetic predisposition to psychopathology. J.Autism Dev.Disord. 1988; 18:343–354. [PubMed: 3170453]
6. Lachiewicz AM, Spiridigliozzi GA, Gullion CM, Ransford SN, Rao K. Aberrant behaviors of young boys with fragile X syndrome. Am J Ment Retard. 1994; 98:567–579. (1994). [PubMed: 8192902]
7. Einfeld S, Tonge B, Turner G. Longitudinal course of behavioral and emotional problems in fragile X syndrome. Am J Med Genet. 87:436–439. 199. [PubMed: 10594885]

8. Rogers SJ, Wehner DE, Hagerman R. The behavioral phenotype in fragile X: symptoms of autism in very young children with fragile X syndrome, idiopathic autism, and other developmental disorders. J.Dev Behav.Pediatr. 2001; 22:409–417. [PubMed: 11773805]
9. Giedd JN, Blumenthal J, Jeffries NO, Castellanos FX, Liu H, Zijdenbos A, Paus T, Evans AC, Rapoport JL. Brain development during childhood and adolescence: a longitudinal MRI study. Nature Neuroscience. 1999; 2(10):861–863.
10. Hazlett HC, Poe MD, Lightbody AA, Gerig G, MacFall JR, Ross AK, Provenzale J, Martin S, Reiss AL, Piven J. Teasing apart the heterogeneity of autism: Same behavior, different brains in toddlers with autistic disorder with and without fragile X syndrome. J Neurodev Disorders. 2009; 1:81–90.
11. Hoeft F, Lightbody AA, Hazlett HC, Patnaik S, Piven J, Reiss A. 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. 2008. [PubMed: 18762595]
12. Hoeft F, Carter JC, Lightbody AA, Hazlett HC, Piven J, Reiss A. Region-specific alterations in brain development in one- to three-year-old boys with fragile X syndrome. Proc Natl Acad Sci. 2010; 107:9335–9339. [PubMed: 20439717]
13. Hoeft F, Walter E, Lightbody AA, Hazlett HC, Chang C, Piven J, Reiss AL. Neuroanatomical differences in toddler boys with fragile X syndrome and idiopathic autism. Arch Gen Psych. 2011; 68(3):295–305.
14. Mesibov GB, Schopler E, Schaffer B, Michal N. Use of the childhood autism rating scale with autistic adolescents and adults. J Am Acad Child Adol Psychiatry. 1989; 28(4):538–541.
15. Willemsen R, Mohkamsing S, de Vries B, Devys D, van den Ouweland A, Mandal JL, Jaljaard H, Oostra B. Rapid antibody test for fragile X syndrome. Lancet. 1995; 345:1147–1148. [PubMed: 7723547]
16. Hazlett HC, Poe MD, Gerig G, Smith RG, Provenzale J, Ross A, et al. Magnetic resonance imaging and head circumference study of brain size in autism: birth through age 2 years. Arch Gen Psychiatry. 2005; 62:1366–1376. (2005). [PubMed: 16330725]
17. Lord C, Rutter M, LeCouteur 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):659–685. [PubMed: 7814313]
18. DiLavore PC, Lord C, Rutter M. The pre-linguistic autism diagnostic observation schedule. J Autism Dev Disord. 1995; 25(4):355–379. [PubMed: 7592249]
19. Mullen EM. Mullen Scales of Early Learning AGS Edition. American Guidance Service, Inc. 1995
20. Elliott, C. The Psychological Corporation. 2nd Ed (DAS-II). San Antonio, TX: 2006. The Differential Abilities Scale.
21. Sparrow, SS.; Balla, DA.; Cicche, HV. Vineland Adaptive Behavior Scales- Interview Edition Survey Form Manual. American Guidance Service, Inc; Circle Pines: 1984.
22. Aman MG, Burrow WH, Wolford PL. The Aberrant Behavior Checklist- Community: factor validity and effect of subject variables for adults in group homes. Am J Ment Retard. 1995; 100(3):283–292. [PubMed: 8554775]
23. Bodfish J, Symons F, Lewis M. The Repetitive Behavior Scales. Western Carolina Center Research Reports. 1999
24. Van Leemput K, Maes F, Vandermeulen D, Suetens P. Automated model-based bias field correction of MR images of the brain. IEEE Transactions on Medical Imaging. 1999; 18:885–896. [PubMed: 10628948]
25. Van Leemput K, Maes F, Vandermeulen D, Suetens P. Automated model-based tissue classification of MR images of the brain. IEEE Transactions on Medical Imaging. 1999; 18:897–908. [PubMed: 10628949]
26. Reiss AL, Abrams MT, Greenlaw R, Freund L, Denckla MB. Neurodevelopmental effects of the FMR-1 full mutation in humans. Nat Med. 1995; 1:159–167. (1995). [PubMed: 7585014]
27. Hessl D, Rivera SM, Reiss AL. The neuroanatomy and neuroendocrinology of fragile X syndrome. Ment Retard Dev Disabil Res Rev. 2004; 10:17–24. [PubMed: 14994284]

28. Hall SS, Lightbody AA, Hirt M, Rezvani A, Reiss AL. Autism in Fragile X Syndrome: A category mistake? J Am Acad Child Adolesc Psychiatry. 2010; 49(9):921–933. [PubMed: 20732628]

29. Baranek GT, Danko CD, Skinner ML, Bailey DB, Hatton DD, Roberts JE, Mirrett PL. Video analysis of sensory-motor features of infants with fragile X syndrome at 9-12 months of age. J Autism Dev Disord. 2005; 35(5):645–656. [PubMed: 16172809]

30. Harlow EG, Till SM, Russell TA, Wijutunge LS, Kind P, Contractor A. Critical period plasticity is disrupted in the barrel cortex of FMR1 knockout mice. Neuron. 2010; 65(3):385–398. [PubMed: 20159451]

31. Bassell GJ, Warren ST. Fragile X syndrome: loss of local mRNA regulation alters synaptic development and function. Neuron. 2008; 60:201–214. [PubMed: 18957214]

32. Saffary R, Xie Z. FMRP regulates the transition from radial glial cells to intermediate progenitor cells during neocortical development. J Neurosci. 2011; 31(4):1427–1439. [PubMed: 21273427]

33. Polleux F, Whitford KL, Kijkhuizen PA, Vitalis T, Ghosh A. Control of cortical interneuron migration by neurotrophins and PI3-kinase signaling. Development. 2002; 129(13):3147–3160. [PubMed: 12070090]

34. Louhivuori V, Vicario A, Uutela M, Rantamaki T, Louhivuori LM, Castren E, Tongiorgi E, Akerman KE, Castren ML. BDNF and DrkB in neuronal differentiation of Fmr1-knockout mouse. Neurobiology Disease. 2011; 41:469–480.

35. Grossman AW, Aldridge GM, Lee KJ, Zeman NK, Jun CS, Azam HS, Arii T, Imoto K, Greenough WT, Rhyu IJ. Developmental characteristics of dendritic spines in the dentate gyrus of Fmr1 knockout mice. Brain Res. 2010; 1355:221–227. [PubMed: 20682298]

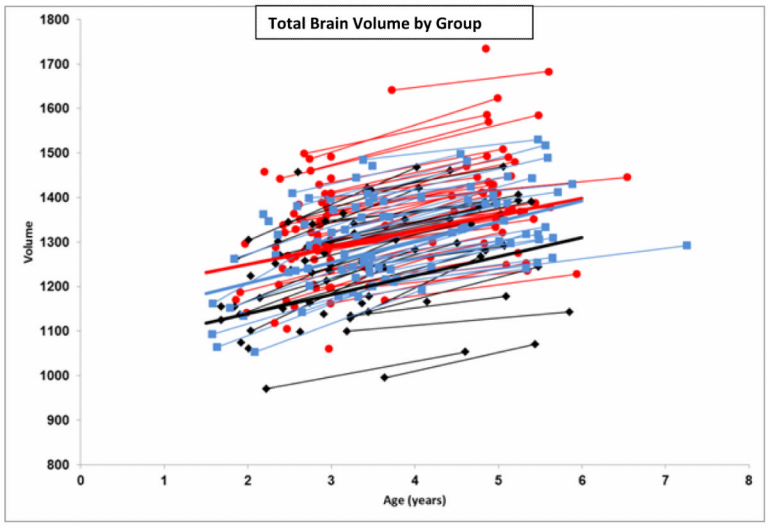

Figure 1.
Longitudinal trajectories for total brain volume (TBV) in between children with fragile X syndrome (FXS), idiopathic autism (iAUT), and controls. Note: Red = iAUT; Blue = FXS; Black = Controls.

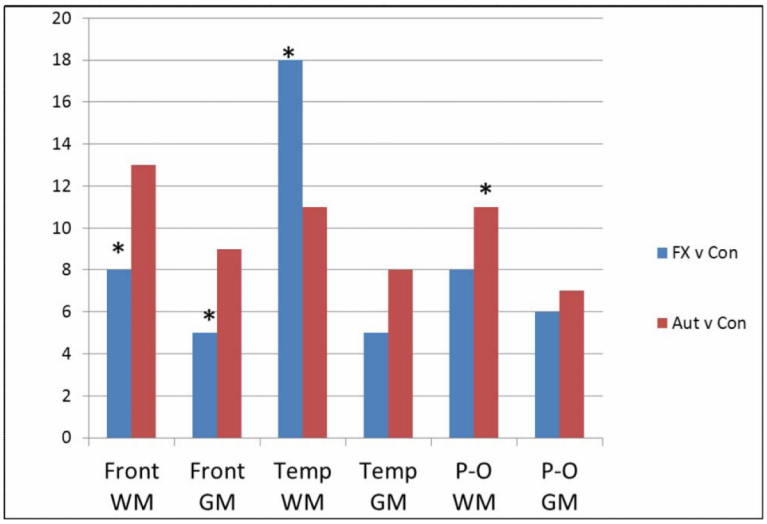

Figure 2.

Percent increase in cortical lobe tissue volumes in Fragile X Syndrome (FXS) and idiopathic autism (iAUT) groups compared to controls (represented by x axis). Note: Percent differences between FXS and iAUT groups compared to controls (y axis) are shown after adjusting age and IQ. * indicates comparisons significant at $p<.05$ after controlling for total brain volume (TBV), age, and IQ.

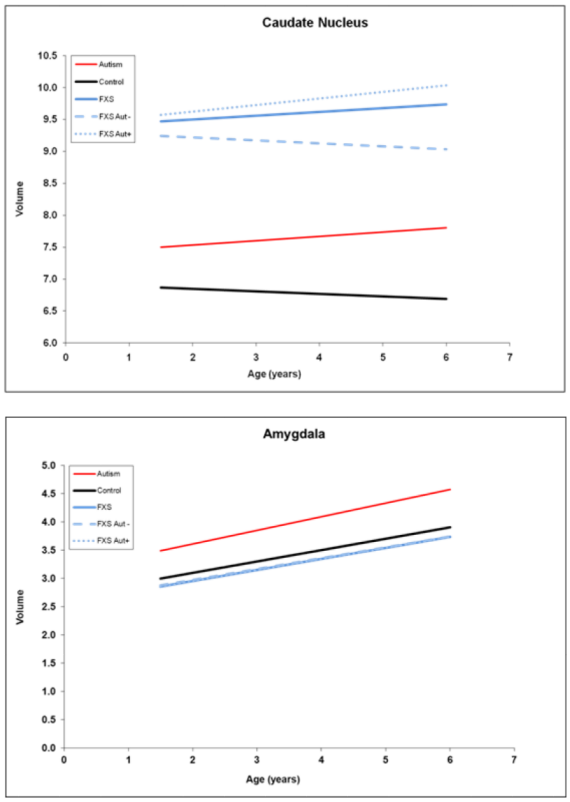

Figure 3.
Trajectories for group differences in caudate and amydala volumes.

Table 1

Sample Descriptives.

Group	Time 1 n	Age (yrs) M (SD)	Time 2 n	Age (yrs) M (SD)
Fragile X Syndrome	53	2.9 (.62)	39	4.92 (.80)
Autism Spectrum Disorder	68	2.79 (.39)	44	5.04 (.43)
Typically Developing	31	2.55 (.59)	19	4.59 (.51)
Developmental Delay	19	2.96 (.50)	7	5.13 (.62)

Note: All subjects were male. yrs = years.

Table 2

Cognitive and adaptive functioning of subjects

		Mullen[a]	Vineland[b]
Group	**n**	**M (SD)**	**M (SD)**
FXS			
time 1	53	54.87 (9.07)	62.13 (9.74)
time 2	39	52.21 (9.85)	51.33 (8.87)
ASD			
time 1	68	54.97 (10.98)	62.74 (8.50)
time 2	44	63.23 (22.57)	56.74 (15.21)
TYP			
time 1	31	109.55 (17.24)	97.20 (12.67)
time 2	19	112.89 (14.76)	93.83 (11.97)
DD			
time 1	19	55.47 (7.53)	64.16 (11.73)
time 2	7	58.86 (11.22)	56.43 (13.75)

Note: ASD = autism spectrum disorder; DD = developmental delay; FXS = Fragile X Syndrome; TYP = typically developing children.

[a] Mullen Scales of Early Learning (MSEL) Early Learning Composite (ELC) Standard Score

[b] Vineland Adaptive Behavior Composite (VABS) Standard Score

Table 3

Raw means for brain volumes for Fragile X Syndrome (FXS), Combined Control Group, Typically Developing Controls (TYP), and Developmentally Delayed Controls (DD) at two timepoints

Volume (cm^3)	Time[a]	FXS		Controls		TYP		DD	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total Brain Volume	1	1282.2	102.1	1208.6	130.4	1230.3	112.9	1172.4	151.9
	2	1350.1	91.5	1316.3	111.7	1340.0	105.0	1251.9	111.1
Total Tissue Volume	1	1155.3	93.1	1091.9	115.8	1111.0	96.9	1060.0	139.0
	2	1223.0	83.2	1202.4	99.7	1218.3	92.2	1159.0	113.8
Total Gray Matter	1	832.2	66.1	789.8	82.9	803.9	67.8	766.5	101.2
	2	869.3	57.0	861.8	74.1	872.8	65.8	832.1	92.2
Total White Matter	1	323.1	30.1	302.1	34.5	307.1	30.8	293.6	39.2
	2	353.7	29.8	340.5	29.3	345.6	30.2	326.9	23.3
Cerebral Spinal Fluid	1	126.9	24.8	116.7	26.4	119.3	25.1	112.4	28.7
	2	127.1	26.4	114.0	30.0	121.7	28.3	93.0	25.5
Cerebellum	1	121.0	8.9	114.8	13.8	116.7	10.6	111.4	17.8
	2	126.4	11.7	126.6	15.6	127.7	13.5	123.3	22.4
Gray	1	103.1	8.0	96.9	12.0	98.9	9.4	93.5	15.2
	2	107.1	10.6	106.4	12.4	107.7	10.8	102.2	17.2
White	1	18.0	1.7	17.9	2.5	17.9	1.9	17.9	3.2
	2	19.4	1.8	20.3	3.7	20.0	3.0	21.0	5.6
Cerebral Cortex	1	934.8	74.4	891.3	99.1	908.9	82.9	861.1	118.5
	2	1000.5	61.4	986.6	93.4	1007.5	82.1	923.6	106.0
Gray	1	671.2	51.9	644.6	70.9	657.7	57.4	622.3	86.6
	2	712.3	45.5	702.8	69.2	717.4	59.3	658.8	85.1
White	1	263.6	24.4	246.6	29.7	251.2	26.8	238.8	33.5
	2	288.2	20.3	283.8	26.4	290.1	25.3	264.7	21.5
Parietal-Occipital	1	128.4	11.3	169.6	76.7	172.8	77.3	164.1	75.8
	2	131.6	9.5	185.3	80.0	94.1	40.3	176.7	79.5
Gray	1	89.7	39.9	121.5	14.1	123.9	11.5	117.4	17.1

Volume (cm^3)	Time[a]	FXS		Controls		TYP		DD	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
	2	93.1	39.4	131.1	13.9	133.0	12.5	125.3	16.8
White	1	50.9	5.1	48.1	5.5	48.9	4.5	46.7	6.7
	2	54.5	4.8	54.2	5.0	55.2	4.8	51.4	4.7
Temporal	1	77.3	7.4	93.5	56.2	95.4	57.1	90.1	54.9
	2	83.0	6.4	104.2	61.0	106.9	62.3	96.1	57.6
Gray	1	50.1	27.9	74.1	8.6	75.7	7.3	71.3	10.1
	2	54.1	29.4	81.7	8.9	83.8	8.0	75.5	8.9
White	1	22.8	2.7	19.4	2.6	19.8	2.4	18.8	2.7
	2	25.2	2.6	22.5	2.6	23.1	2.7	20.7	1.0
Frontal	1	123.6	11.4	169.3	72.6	172.7	73.5	163.6	71.3
	2	132.4	10.0	188.9	75.0	193.4	76.2	175.4	71.6
Gray	1	88.4	36.4	119.2	13.6	121.7	11.4	115.0	16.0
	2	96.4	37.1	130.4	13.1	133.5	10.8	120.9	15.3
White	1	53.2	6.2	50.1	7.2	51.0	6.7	48.6	7.5
	2	60.3	6.0	58.6	6.0	59.9	5.7	54.5	5.1

[a]Note: Time 1 is approximately 2–3 years of age; Time 2 was approximately 4–5 years of age.

Table 4

Group comparisons for Fragile X Syndrome (FXS) versus controls for global and lobar brain volumes

Volume	Comparisons (FXS vs. Controls)			
	Diff	SE	p	%
Total Brain Volume	73.2	26.3	.006	6%
Total Tissue Volume	64.6	23.5	.007	6%
Total Gray Matter	43.2	16.9	.012	6%
Total White Matter	21.4	7.0	.003	7%
Cerebral Spinal Fluid	8.6	5.2	.10	7%
Cerebellum	0.41	2.2	.85	0%
Gray	1.44	1.9	.45	1%
White	−1.03	0.4	.02	−5%
Cerebral Cortex	−3.01	4.1	.46	0%
Gray	−7.4	3.3	.03	−1%
White	4.38	2.3	.06	2%
Parietal-Occipital	−1.5	3.1	.63	0%
Gray	−2.1	2.3	.34	−1%
White	0.7	1.4	.63	1%
Temporal	2.3	2.1	.28	1%
Gray	−1.9	1.7	.27	−1%
White	4.2	0.8	<.001	10%
Frontal	−5.9	3.5	.09	−2%
Gray	−6.1	2.6	.02	−2%
White	0.2	1.5	.90	0%

Note: Table 4 presents the group differences (Diff) and standard error (SE) for total and lobar brain volumes at 3.5 years of age (the approximate mean age for our sample) after adjusting for age and IQ. Regional comparisons (cerebellum, cerebral cortical volume, cortical lobes) are show with adjustments controlling for age, IQ, and total brain volume (TBV).

Table 5

Group comparisons for selected substructure volumes controlling for age, IQ, and total brain volume (TBV).

Region	FXS v Controls Diff (SE), % diff	FXS v DD Diff (SE), % diff	FXS v TYP Diff (SE), % diff	FXS v iAUT Diff (SE), % diff
Caudate nucleus				
Total	2.42 (.3), 34% ***	2.45 (.4), 35% ***	2.38 (.4), 33% ***	2.14 (.3), 29% ***
Right	1.20 (.2),33% ***	1.21 (.2), 34% ***	1.18 (.2), 32% ***	1.07 (.1), 29% ***
Left	1.22 (.2), 35% ***	1.24 (.2), 36% ***	1.20 (.2), 34% ***	1.06 (.1), 29% ***
Putamen				
Total	0.45 (.2), 5% *	0.35 (.3), 4%	0.56 (.2), 7% *	0.50 (.2), 6% **
Right	0.24 (.1), 6% *	0.19 (.1), 5%	0.29 (.1), 7% *	0.28 (.1), 7% ***
Left	0.22 (.1), 5% *	0.16 (.1), 4%	0.27 (.1), 6% *	0.22 (.1), 5% **
Globus pallidus				
Total	0.41 (.1), 15% ***	0.44 (.1), 16% ***	0.39 (.1), 14% ***	0.22 (.1), 7% **
Right	0.21 (.1), 15% ***	0.23 (.1), 17% ***	0.19 (.1), 13% ***	0.14 (.04), 10% ***
Left	0.21 (.1), 15% ***	0.21 (.1), 15% **	0.20 (.1), 15% ***	0.08 (.04), 5%
Amygdala				
Total	−0.30 (.1), −9% *	−0.39 (.2), −11% *	−0.21 (.2), −6%	−0.66 (.1), −17% ***
Right	−0.16 (.1), −9% *	−0.18 (.1), −10% *	−0.13 (.1), −8%	−0.37 (.1), −18% ***
Left	−0.14 (.1), −8% *	−0.21 (.1), −11% *	−0.08 (.1), −5%	−0.29 (.1), −16% ***
Hippocampus				
Total	0.23 (.2), −4%	0.23 (.2), −4%	−0.23 (.2), −4%	−0.34 (.1), −6% **
Right	0.04 (.1), −1%	−0.06 (.2), −2%	−0.01 (.1), 0%	−0.07 (.1), −2%
Left	0.19 (.1), −7%	−0.17 (.1), −6%	−0.21 (.1), −7% *	−0.28 (.1), −9% ***

Note: Fragile X Syndrome (FXS) group refers to entire sample of children with FXS. The Control group contained both developmentally delayed (DD) and typically developing (TYP) subjects, but comparisons with the DD and TYP subgroups are also displayed separately. Diff = group differences; iAUT = idiopathic autism; SE = standard error.

* $p \leq .05$

** $p \leq .01$

*** $p \leq .001$

Table 6

Autism Diagnostic Interview–Revised (ADI-R) subdomain scores for Fragile X Syndrome (FXS) in the subgroup with autism (FXS+A), without autism (FXS-A), and the autism spectrum disorder (ASD) group

ADI-R subdomain	FXS+Aut M(SD)	FXS-A M(SD)	ASD M (SD)
Communication			
Verbal	n=9	n=17	n=58
	8.33 (3.4)	8.47 (3.5)	9.47 (2.6)
Nonverbal	n=14	n=25	n=60
	10.43 (1.9)	6.48 (3.8)	10.73 (2.2)
Social	17.47 (3.8)	9.87 (5.8)	18.13 (6.2)
Ritualistic-repetitive	4.87 (1.8)	3.13 (1.8)	4.93 (1.8)

Table 7

Comparisons for Fragile X Syndrome (FXS) subgroups compared to idiopathic autism (iAUT) and each other for selected substructure volumes after controlling for age, IQ, and total brain volume (TBV). Comparisons for Fragile X Syndrome with autism (FXS+Aut) vs. Fragile X Syndrome without autism (FXS-Aut) are shown after controlling for age and TBV only.

Region	FXS+Aut vs. iAUT Diff (SE), % diff	FXS-Aut vs. iAUT Diff (SE), % diff	FXS+Aut vs. FXS-Aut Diff (SE), % diff
Caudate nucleus			
Total	2.31 (.3), 31% ***	1.73 (.3), 23% ***	.63 (.4), 7%
Right	1.16 (.2), 31% ***	0.88 (.1), 24% ***	.29 (.2), 6%
Left	1.16 (.2), 32% ***	0.84 (.1), 23% ***	.33 (.2), 7%
Putamen			
Total	0.43 (.2), 5% *	0.35 (.3), 4%	−.27 (.3), −3%
Right	0.24 (.1), 6% *	0.19 (.1), 5%	−.15 (.1), −3%
Left	0.19 (.1), 5%	0.16 (.1), 4%	−.12 (.2), −3%
Globus pallidus			
Total	0.26 (.1), 9% ***	0.12 (.1), 4%	.13 (.1), 4%
Right	0.16 (.05), 11% ***	0.08 (.04), 6% *	.07 (.1), 5%
Left	0.10 (.1), 7%	0.04 (.04), 2%	.06 (.1), 4%
Amygdala			
Total	−0.68 (.1), −17% ***	−0.63 (.1), −11% ***	−.01 (.2), 0%
Right	−0.38 (.1), −19% ***	−0.34 (.1), −10% ***	−.02 (.1), −1%
Left	−0.30 (.2), −16% ***	−0.29 (.1), −11% ***	.01 (.1), 1%
Hippocampus			
Total	−0.39 (.2), −7% *	−0.17 (.2), −3%	−.17 (.2), −3%
Right	−0.08 (.1), −3%	−0.05 (.1), −2%	−.05 (.1), −2%
Left	−0.31 (.1), −11% ***	−0.12 (.1), −4%	−12 (.1), −4%

Note: Diff = group differences; SE = standard error.

* p ≤ .05

** p ≤ .01

*** p ≤ .001