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Neuroanatomical Differences in Toddler Boys With Fragile X Syndrome and Idiopathic Autism

Fumiko Hoeft, MD, PhD; Elizabeth Walter, PhD; Amy A. Lightbody, PhD; Heather C. Hazlett, PhD; Catie Chang, MS; Joseph Piven, MD; Allan L. Reiss, MD

Context: Autism is an etiologically heterogeneous neurodevelopmental disorder for which there is no known unifying etiology or pathogenesis. Many conditions of atypical development can lead to autism, including fragile X syndrome (FXS), which is presently the most common known single-gene cause of autism.

Objective: To examine whole-brain morphometric patterns that discriminate young boys with FXS from those with idiopathic autism (iAUT) as well as control participants.

Design: Cross-sectional, in vivo neuroimaging study.

Setting: Academic medical centers.

Patients: Young boys (n=165; aged 1.57-4.15 years) diagnosed as having FXS or iAUT as well as typically developing and idiopathic developmentally delayed controls.

Main Outcome Measures: Univariate voxel-based morphometric analyses, voxel-based morphometric multivariate pattern classification (linear support vector machine), and clustering analyses (self-organizing map).

Results: We found that frontal and temporal gray and white matter regions often implicated in social cognition, including the medial prefrontal cortex, orbitofrontal cortex, superior temporal region, temporal pole, amygdala, insula, and dorsal cingulum, were aberrant in FXS and iAUT as compared with controls. However, these differences were in opposite directions for FXS and iAUT relative to controls; in general, greater volume was seen in iAUT compared with controls, who in turn had greater volume than FXS. Multivariate analysis showed that the overall pattern of brain structure in iAUT generally resembled that of the controls more than FXS, both with and without AUT.

Conclusions: Our findings demonstrate that FXS and iAUT are associated with distinct neuroanatomical patterns, further underscoring the neurobiological heterogeneity of iAUT.

Arch Gen Psychiatry. 2011;68(3):295-305.

Published online November 1, 2010.

doi:10.1001/archgenpsychiatry.2010.153

Author Affiliations: Center for Interdisciplinary Brain Sciences Research, Department of Psychiatry and Behavioral Sciences (Drs Hoeft, Walter, Lightbody, and Reiss) and Department of Radiology (Ms Chang), School of Medicine, and Department of Electrical Engineering (Ms Chang), Stanford University, Stanford, California; and Carolina Institute for Developmental Disabilities, University of North Carolina, Chapel Hill (Drs Hazlett and Piven).

CURRENTLY, LISTS OF INCLUSION and exclusion criteria form the basis of all DSM-based diagnoses. One prevalent developmental disorder, autism (AUT), is characterized by a suite of altered behaviors including difficulties with social interactions, impairments in language, and repetitive

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and restrictive interests.¹ Interestingly, many individuals with fragile X syndrome (FXS), a condition arising from mutations of a specific gene on the X chromosome, also exhibit behaviors on the AUT spectrum, making FXS the most common known single-gene cause of AUT. Be-

cause of the broad similarity in behavioral phenotype, researchers have hoped that characterization of the morphological brain changes in FXS may lead to a helpful neuroanatomical model for idiopathic AUT (iAUT) as well. However, aberrant behaviors are likely the result of a complex interplay of brain changes, and the correspondence between behavior and brain change may not necessarily be one-to-one. That is, a behavior that looks similar to an outside observer may potentially be caused by any of a number of different brain states. There is still little evidence supporting the idea that the similarly aberrant behaviors exhibited by those with FXS and iAUT are the result of similar brain changes. Thus, it is possible that the behaviors exhibited by FXS and iAUT, although similar on the surface, are the re-

sult of differing morphological brain changes. Although we are operating within the framework just outlined, the utility and validity of the diagnostic taxonomy of AUT and the (dis)similarities between symptoms of AUT seen in FXS and iAUT are currently topics of active discussion.²

Two recent studies have directly compared the brains of individuals with FXS and iAUT. One study performed by our group examined gray matter volume (GMV) of a small number of a priori selected subcortical and mesial temporal brain regions of interest in the same sample as our current study: a large sample of very young boys with FXS and iAUT as well as typically developing (TD) boys and those with idiopathic developmental delay (DD). This previous study found that the amygdala-caudate profile distinguished individuals with iAUT from those with FXS (both with and without AUT). Specifically, those with iAUT were found to have a larger amygdala, while those with FXS had a larger caudate.³ In another study, voxel-based morphometry (VBM) of GMV was performed in a small number of adults with FXS, adults with iAUT, and TD control adults (N=30).⁴ Compared with participants with iAUT and controls, those with FXS had greater dorsolateral prefrontal cortex and caudate volumes and reduced volumes in the left post-central, middle temporal, and right fusiform gyri. As compared with participants with FXS and controls, those with iAUT had smaller cerebellar volumes.

Although these results are intriguing, the current study extends the previous findings in 4 novel ways: (1) we examine both GMV and white matter volume (WMV) in a large number of very young children with FXS, those with iAUT, TD children, and DD children, which is important as white matter differences are thought to play an important role in AUT⁵; (2) we examine the whole brain, relative to previous studies that have typically restricted their analyses using volumetric measures or small-volume correction to a priori hypothesized regions; (3) we examine morphometric patterns in which FXS and iAUT are on opposite extremes of controls, ie, FXS > controls > iAUT and iAUT > controls > FXS (findings from this analysis are particularly novel as they demonstrate that diametrically differing neuroanatomical patterns can lead to similar symptoms, ie, 2 sides of the same coin)³; and (4) we combine univariate VBM and multivariate supervised and unsupervised machine learning algorithms to identify fine-grained patterns that differentiate between groups.^{6,7} We find that results from univariate and multivariate analyses are largely complementary; univariate analysis examines between-group differences in voxel intensities (volumes) 1 voxel at a time, whereas multivariate pattern classification analysis (MVPA) identifies patterns of voxel intensities that are different (or discriminate) between groups and does not require individual voxels to be different.⁸⁻¹⁰

We hypothesized that if iAUT and FXS are indeed neuroanatomically distinct, as some studies are beginning to suggest, there should be little overlap in the abnormal brain morphometric patterns that distinguish iAUT or FXS from TD and DD controls and the discrimination accuracy using MVPA between iAUT and FXS should be high. If, on the other hand, FXS is a rep-

resentative neuroanatomical model for iAUT, then discrimination between iAUT and FXS using morphometric pattern classification algorithms would be poor and there should be considerable overlap in the spatial patterns of brain abnormalities found in both iAUT and FXS as compared with TD and DD controls. Further, as is increasingly suggested by studies in myriad disciplines,¹¹ iAUT may comprise many currently unidentified subgroups with diverse etiologies and disease pathways. If this is the case and iAUT is indeed etiologically heterogeneous, one may hypothesize that participants with iAUT as a group will be more similar to TD and DD controls, who are also neurobiologically heterogeneous as groups, as opposed to individuals composing the FXS group who share the same genetic risk factor for aberrant neurodevelopment.

METHODS

PARTICIPANTS

Participants for this study were recruited by collaborating research teams at the Stanford University School of Medicine and the University of North Carolina, Chapel Hill. The study protocols were approved by the human subjects committees at the Stanford University School of Medicine and the University of North Carolina, Chapel Hill, and consent was obtained. The TD children (n=31; mean [SD] age, 2.55 [0.60] years) and DD children (n=19; mean [SD] age, 2.96 [0.50] years) were recruited through local intervention programs, preschools, child care facilities, community media, and state-run agencies (eg, Regional Center system in California and Child Development Service Agencies in North Carolina). Children with FXS (n=52; mean [SD] age, 2.90 [0.63] years) were recruited through registry databases maintained by the Stanford University School of Medicine and the University of North Carolina, Chapel Hill, through postings to the National Fragile X Foundation Web site and quarterly newsletter, and through mailings to other regional FXS organizations. Children with iAUT (n=63; mean [SD] age, 2.77 [0.41] years) were recruited from clinics specializing in pervasive developmental disorders in North Carolina and from community clinics and service agencies for the Stanford University School of Medicine site (**Table 1**, eTable 1, eTable 2, and eFigure 1 [<http://www.archgenpsychiatry.com>] include demographic characteristics, cognitive abilities, and brain tissue volumes). Participants with FXS and iAUT were tested with the Autism Diagnostic Interview (ADI)-Revised¹² and the Autism Diagnostic Observation Schedule-Generic (ADOS).¹³⁻¹⁵

Children were included in the iAUT group if they had received a clinical diagnosis of AUT and met all criteria on the ADI-Revised and/or the ADOS. Participants were excluded from the study if they were born preterm (<34 weeks), had a low birth weight (<2000 g), showed evidence of a genetic condition or syndrome other than FXS, exhibited sensory impairments, or had any serious medical or neurological condition that affected growth or development (eg, seizure disorder, diabetes, congenital heart disease). Further, the FXS group was divided into subgroups based on their scores on the ADOS and the ADI at the time of their scan. Those children who met full criteria for AUT on both the ADOS and the ADI were placed in the FXS with AUT (FXS+A) group. Children who did not meet full criteria on these 2 measures were placed in the FXS without AUT (FXS-A) group. Details regarding demographic information and the distribution of the sample between recruit-

Table 1. Demographic Information

Characteristic	TD	DD	FX	iAUT	ANOVA		Post Hoc P Value
					F Score	P Value	
Participants at SU:UNC, No. ^a	11:20	11:8	28:24	17:46	11.32	.01	
Age, y							
Participants, No.	31	19	52	63			FX>TD ^b , DD>TD ^b
Mean (SD)	2.55 (0.60)	2.96 (0.50)	2.90 (0.63)	2.77 (0.41)	3.65	.01	
Mullen Scales of Early Learning composite standard score							
Participants, No.	31	19	52	63			FX<TD ^c , iAUT<TD ^c , DD<TD ^c
Mean (SD)	109.55 (17.24)	55.47 (7.53)	54.94 (9.14)	54.10 (9.41)	207.13	<.001	
RBS overall total score							
Participants, No.	16	14	37	16			FX>TD ^c , iAUT>TD ^c , >DD ^b
Mean (SD)	3.13 (4.27)	13.07 (12.42)	18.70 (11.08)	26.25 (14.36)	12.69	<.001	
ADI repetition subscale total score							
Participants, No.	NA	NA	50	63			
Mean (SD)	NA	NA	3.12 (1.49)	4.84 (1.76)	30.39	<.001	
ADI socialization subscale total score							
Participants, No.	NA	NA	50	63			
Mean (SD)	NA	NA	9.18 (4.92)	18.62 (4.04)	125.53	<.001	
ADI verbal communication subscale score							
Participants, No.	NA	NA	5	5			
Mean (SD)	NA	NA	6.80 (5.07)	12.80 (2.59)	5.56	.05	
ADI nonverbal communication subscale score							
Participants, No.	NA	NA	45	58			
Mean (SD)	NA	NA	9.09 (3.74)	11.59 (2.12)	18.23	<.001	
ADI verbal communication sum of scores							
Participants, No.	NA	NA	5	5			
Mean (SD)	NA	NA	18.20 (10.92)	35.60 (4.16)	11.09	.01	
ADI nonverbal communication sum of scores							
Participants, No.	NA	NA	44	58			
Mean (SD)	NA	NA	26.43 (8.90)	39.52 (5.78)	80.63	<.001	
Adjusted ADI sum of scores							
Participants, No.	NA	NA	49	63			
Mean (SD)	NA	NA	0.93 (0.36)	1.43 (0.23)	81.53	<.001	
ADOS socialization and communication total score							
Participants, No.	NA	NA	52	54			
Mean (SD)	NA	NA	10.19 (5.71)	18.00 (2.87)	80.05	<.001	
ADOS severity measure score							
Participants, No.	NA	NA	52	53			
Mean (SD)	NA	NA	4.10 (2.38)	7.62 (1.43)	85.12	<.001	
FMRP							
Participants, No.	NA	NA	50	NA			
Mean (SD), %	NA	NA	5.83 (3.94)	NA			

Abbreviations: ADI, Autism Diagnostic Interview; ADOS, Autism Diagnostic Observation Schedule, Repetitive and Stereotype; DD, idiopathic developmental delay; FMRP, fragile X mental retardation protein; FXS, fragile X syndrome; iAUT, idiopathic autism; NA, not applicable; RBS, Repetitive Behavior Scale; SU, Stanford University School of Medicine; TD, typically developing; UNC, University of North Carolina, Chapel Hill.

^aPearson χ^2 performed. No significant difference in any of the measures between sites (SU and UNC) for each diagnostic group.

^bSignificant at $P < .05$.

^cSignificant at $P < .001$.

ment sites can be found in Table 1 and eTable 2. There were no significant differences between sites in any of the cognitive measurements for each diagnostic group (all $P > .05$).

The eAppendix includes further information regarding our methods related to genotyping, cognitive measures, neuropsychiatric assessments, magnetic resonance imaging, preprocess-

ing procedures, and cross-site validation of imaging parameters.

UNIVARIATE ANALYSES OF MAGNETIC RESONANCE IMAGES USING GENERALIZED LINEAR MODELS

Regional GMV and WMV differences between FXS, iAUT, and controls (TD and DD combined) were examined using whole-brain analysis of covariance, covarying out age, site, and total GMV or total WMV for gray matter and white matter analyses, respectively. We used 2 control groups as TD represents typical development and DD allows us to better match for overall cognitive functioning (ie, lower overall IQ) as well as for the putative widespread neural effects associated with the presence of a significant developmental disorder. The 2 control samples (TD and DD) were initially grouped together because of the overall small sample size, and the results obtained were subsequently examined separately for the TD and DD groups. The main analyses of interest were the comparisons between FXS and iAUT, FXS and controls, and iAUT and controls. In all VBM analyses, images were thresholded with a joint expected probability threshold of $P < .01$ (height) and $P < .01$ (familywise error corrected for spatial extent), corrected for non-stationary cluster extent threshold (nonisotropic smoothness).¹⁶ Volumes of these significant regions were then extracted and compared separating TD and DD controls and separating children with FXS-A and FXS+A.

Images containing spatial information regarding significant regions were then combined to create overlap maps. These maps display voxels that illustrate relationships between groups, such as regions that significantly differentiate between FXS and TD/DD controls as well as between FXS and iAUT. We also display maps that indicate differences between FXS and controls as well as between iAUT and controls. These regions were extracted individually and correlated with the total Repetitive Behavior Scale score,¹⁷ adjusted ADI sum (corrected for the number of items given to each child), ADOS composite score, ADOS severity score,¹³ and all ADI and ADOS subtests for FXS and iAUT separately.

MVPA OF MAGNETIC RESONANCE IMAGES USING LINEAR SUPPORT VECTOR MACHINE ANALYSES

We performed linear support vector machine (SVM) analyses to identify regions where spatially distributed patterns of GMV and WMV differences were particularly useful in discriminating between groups of participants (eg, between brains of individuals with FXS and iAUT).^{9,10} Linear SVM is a machine-learning approach that attempts to classify items (in this case, GMV and WMV maps) based on a linear separation in (highly) multidimensional space.⁸ The output of an SVM classification includes statistical measures of classification accuracy. In this manner, we can assess the differences and similarities of 2 groups of brains based on how accurately or poorly they can be discriminated with SVM.

Before carrying out SVM analyses, each individual's spatially normalized and modulated but unsmoothed GMV and WMV images were resampled to $4 \times 4 \times 4$ -mm voxels and converted to matrices followed by calculation of the residuals taking age, site, and total GMV or total WMV into account and normalizing the matrix such that mean=0 and SD=1. The SVM analysis between FXS+A and iAUT was also performed on behavioral data alone to examine whether these 2 groups could be distinguished in this manner. Behavioral measures included all subtests and/or composite scores of the ADI, ADOS, Mullen Scales of Early Learning,¹⁸ and Vineland Adaptive Behavior Scales.¹⁹ Behavioral scores

for these measures were available in most subjects. However, when needed, missing values were replaced by the mean of their diagnostic group (data from 2 children with iAUT and none from children with FXS were missing for the Vineland Adaptive Behavior Scales; Table 1 shows this for other measures). When different modules were given and standardized scores were not available, adjusted scores were calculated correcting for the number of items. Wherever indicated in the results as "whole-brain SVM," principal components analysis was performed to reduce the number of dimensions to N eigenvectors, where N was the minimum number of components that accounted for at least 70% of the variance. On some occasions, feature reduction using recursive feature elimination (RFE)^{20,21} was performed (indicated as RFE-SVM), where the bottom 30% of the voxels based on the absolute value of their weights were iteratively excluded until the performance started degrading.

The matrices with vectors for $n-1$ participants (ie, all participants except for 1, out of a matrix comprising 2 groups of participants) were input as a training data set to train a linear support vector pattern classifier (with fixed regularization parameter $C=1$) to correctly identify GMV, WMV, or behavioral patterns of the n^{th} participant. This process of training a classifier and testing on the n^{th} subject was repeated n times until all participants were used as test data once. Unbalanced sample size for the classes was corrected using weighted SVM analysis. Prediction accuracy, sensitivity, specificity, and positive predictive values were calculated.

Analyses were performed with an in-house Matlab-based (Mathworks, Natick, Massachusetts) MVPA toolbox, which adopted LIBSVM.²² The SVM analyses were used to classify FXS from iAUT, FXS+A from iAUT, FXS from TD/DD, iAUT from TD/DD, and TD from DD. In addition, we performed SVM analysis of FXS and TD/DD and applied the resulting classifier to iAUT to determine whether this group would appear more similar to TD/DD or FXS. Further, we repeated analyses including only brain voxels from the bilateral caudate and cerebellar vermis regions to determine whether SVM classification would be altered when the only voxels used for classification were those from brain regions that have been reported to be morphometrically similar between FXS and iAUT.²³ To perform this limited voxel analysis, we coregistered bilateral caudate and vermis regions from the automated anatomical labeling atlas²⁴ to the custom template and extracted GMV values from all subjects' images as described earlier. Classification accuracies were statistically compared using permutation analyses (ie, classes were randomly permuted and analyses were repeated 2000 times to obtain the distribution of data).

Finally, we used self-organizing maps (Neural Network toolbox, Matlab R2009b) to visualize and convert complex relationships between high-dimensional features (voxels) into simple geometric relationships.²¹ The goal was to examine the brain-based representations of iAUT in relation to those of FXS and controls. The default setting was used to train a 2×2 two-dimensional map of 4 neurons (clusters). Prior to training, the number of features (voxels) was reduced using RFE-SVM^{20,21}; this process selected voxels that jointly discriminated between TD/DD and FXS. Note that because the main goal of this analysis was to examine the spatial relationship between iAUT and other groups, this procedure does not bias the results.

RESULTS

BEHAVIORAL RESULTS

Between FXS and iAUT (Table 1), the ADI and ADOS measures of social, communication, and repetitive behavior indicated greater behavioral problems in iAUT as

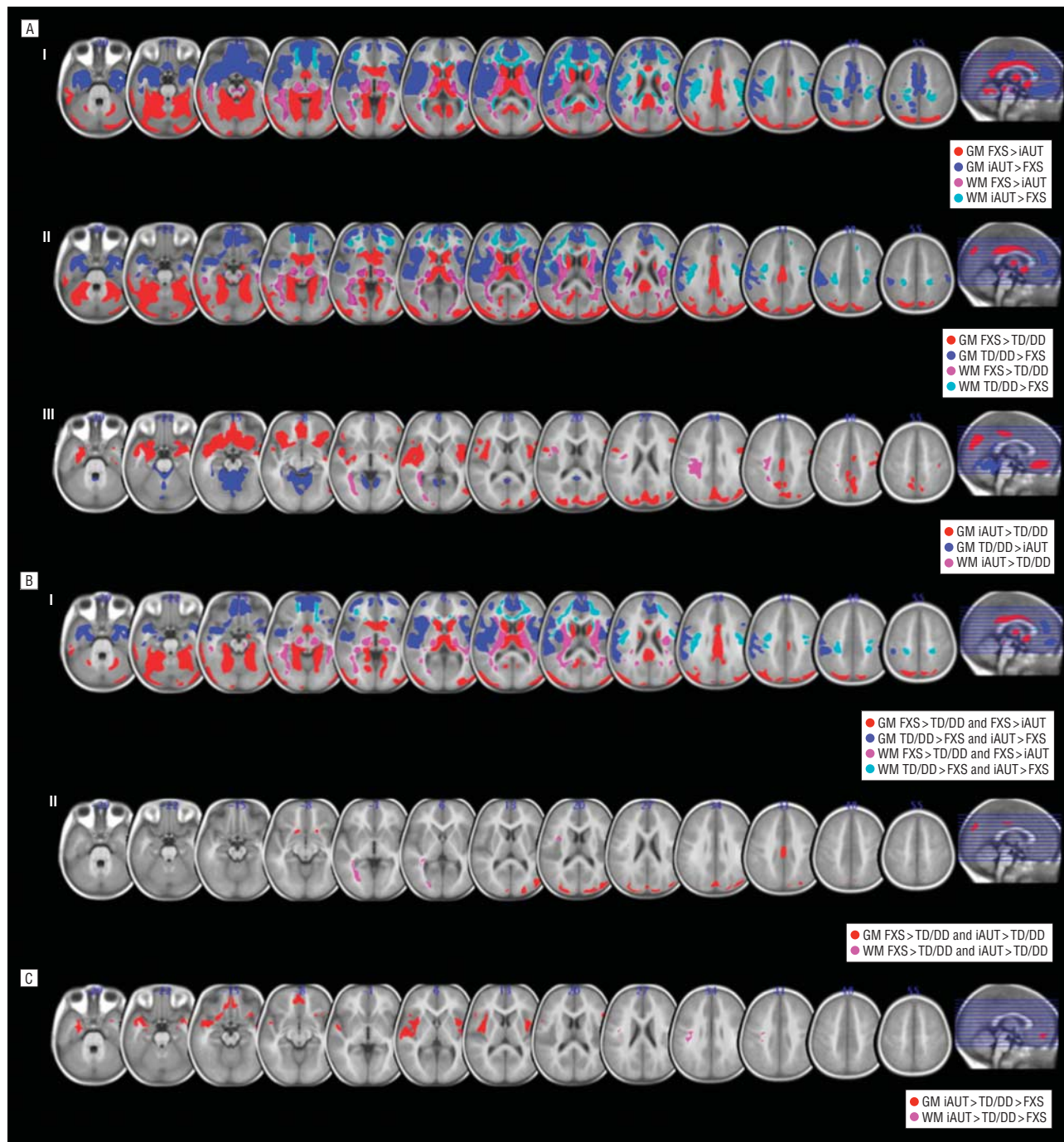


Figure 1. Differences in regional brain volumes between groups. A, Regions showing significant differences in regional gray matter (GM) volume and white matter (WM) volume between fragile X syndrome (FXS) and idiopathic autism (iAUT) (panel I), FXS and typically developing (TD) and idiopathic developmentally delayed (DD) controls (panel II), and iAUT and TD/DD controls (panel III). B, Regions showing similar regional brain volumes for iAUT and TD/DD controls compared with FXS and for TD/DD controls and iAUT compared with FXS (panel I), and regions showing similar regional brain volumes for FXS and iAUT compared with TD/DD controls, overlaid on custom T1 template (panel II). C, Regions showing opposite regional volume patterns for FXS and iAUT. The left side shows the right hemisphere. The statistical threshold is set at $P = .01$, familywise error cluster-level corrected.

compared with FXS. However, repetitive behavior as measured by the Repetitive Behavior Scale as well as IQ were not significantly different between the 2 groups. When FXS+A and FXS-A were compared (eTable 2), all behavioral measures including repetitive behavior and social and communication skills (but not IQ) showed significant between-group differences. As expected, FXS+A showed more severe problems in these domains than did

FXS-A. Scores for FXS+A and iAUT (eTable 2) were fairly similar across domains. While ADI measures of social function were significantly more impaired in iAUT than in FXS+A, the ADOS social and communication scores and ADI communication measures were not significantly different between these groups. Repetitive behavior and IQ were also not significantly different between FXS+A and iAUT (Table 1 and eTable 2).

Table 2. Gray Matter Regions That Show Significant Between-Group Differences in the Univariate Voxel-Based Morphometric Analyses

ROI ^a	Region	Gray Matter Volume						
		BA	Talairach Coordinates			T Score	P Value (Corrected)	Voxels (1 mm ³), No.
			x	y	z			
iAUT > TD/DD								
A	Bilateral cuneus, bilateral posterior cingulate gyrus (parietal and occipital lobes)	19	-19	-85	34	4.57	<.001	21 060
			22	-90	27	4.26		
			2	-23	41	4.19		
B	Bilateral right inferior frontal gyrus, superior temporal gyrus, parahippocampal gyrus (frontal, temporal, and limbic lobes)	38/47	23	21	-16	4.55	<.001	48 448
			33	4	-17	4.45		
			32	15	-10	4.20		
TD/DD > iAUT								
C	Bilateral cerebellar (culmen), fusiform and lingual gyri		12	-51	-3	4.93	<.001	11 630
			-6	-36	-18	3.98		
			-13	-51	0	3.89		
FXS > TD/DD								
F	Bilateral caudate body, bilateral anterior cingulate, bilateral middle cingulate, bilateral posterior cingulate		15	1	20	10.78	<.001	161 001
			12	7	15	10.57		
			-14	6	17	8.68		
TD/DD > FXS								
G	Right orbitofrontal cortex, insula, claustrum, superior parietal cortex (frontal, temporal, and parietal lobes)	13	39	-3	19	8.06	<.001	98 288
			32	5	16	7.94		
			29	15	10	7.89		
H	Left superior temporal gyrus, insula, superior parietal cortex (frontal, temporal, and parietal lobes)	13	-40	-3	20	7.77	<.001	52 800
			-30	14	13	7.45		
			-31	7	16	7.26		

Abbreviations: BA, Brodmann area; DD, idiopathic developmental delay; FXS, fragile X syndrome; iAUT, idiopathic autism; ROI, region of interest; TD, typically developing.

^aCapital letters denote ROIs depicted in eFigure 2A and B, eFigure 3A and B, and eFigure 4A and B.

UNIVARIATE VBM RESULTS

Between-group differences in regional GMV and WMV corrected for total GMV and total WMV, respectively, as well as age and site (the Stanford University School of Medicine and the University of North Carolina, Chapel Hill) are reported in eFigures 1, 2, 3, and 4, **Figure 1**, **Table 2**, and **Table 3**: iAUT vs TD/DD is shown in Figure 1A panel III and eFigure 2, FXS vs TD/DD is shown in Figure 1A panel II and eFigure 3, and FXS vs iAUT is shown in Figure 1A panel I and eFigure 4. Analyses contrasting FXS vs iAUT as well as FXS vs TD/DD show that the morphometric pattern that differentiates FXS from iAUT is qualitatively similar to the pattern that discriminates FXS from TD/DD controls (Figure 1B panel I), implying similar morphometric brain structure across the iAUT and TD/DD groups. Regions composing this morphometric pattern included significantly greater bilateral caudate, thalamus, hypothalamus, parieto-occipital, lingual or fusiform, cerebellar, and cingulate GM regions and perisylvian and temporal WM regions and significantly reduced orbitofrontal cortex, medial prefrontal cortex (mPFC), amygdala, insular, and sylvian GM regions and frontal and sensorimotor WM regions in FXS as compared with iAUT and with TD/DD controls.

While some brain regions showed significant differences in regional volumes between iAUT and TD/DD, these differences were primarily driven by dissimilarity

between iAUT and TD rather than between iAUT and DD (eFigure 2D and E). This is in contrast to brain regions that showed significantly different GMV and WMV between FXS and TD/DD, where FXS was significantly different from both TD and DD groups (eFigure 3E and F). Brain regions differentiating iAUT from TD/DD included significantly greater orbitofrontal cortex, mPFC, amygdala, insular, inferior frontal, parahippocampal, superior temporal sulcus (STS), temporal pole (TP), parieto-occipital, and right temporoparietal GM regions and frontal, sensorimotor, and temporal WM regions and significantly reduced cerebellar and occipital GM regions for iAUT. Notably, there were several brain regions that showed FXS and iAUT to be on the opposite extreme relative to controls, ie, significantly reduced in FXS and increased in iAUT compared with controls, including bilateral STS, TP, orbitofrontal cortex, mPFC, amygdala, insula, and dorsal cingulum (Figure 1C).

We also examined a subset of children with FXS who had a diagnosis of AUT (FXS+A) (eFigure 2D and E, eFigure 3E and F, and eFigure 4E and F). The pattern of differences between FXS and iAUT (ie, brain regions that showed and did not show significant differences between these groups) did not change when FXS+A was compared with iAUT (except for the right dorsal WM in eFigure 2C, region of interest E, eFigure 2E). Finally, we performed regression analyses between the regions detected in these univariate analyses and all domain and

Table 3. White Matter Regions That Show Significant Between-Group Differences in the Univariate Voxel-Based Morphometric Analyses

ROI ^a	Region	White Matter Volume					
		Talairach Coordinates			T Score	P Value (Corrected)	Voxels (1 mm ³), No.
		x	y	z			
iAUT > TD/DD							
D	Near right middle occipital gyrus	35	-54	3	4.09	.009	4033
		26	-79	9	3.79		
		38	-43	5	3.65		
E	Near right frontal lobe and right insula	38	1	21	4.11	.001	6197
		40	-25	33	3.52		
		24	-36	31	3.27		
*	Also found left ROIs mirroring each of these; they failed to reach significance at <i>P</i> = .03						
TD/DD > iAUT							
	Not significant						
FXS > TD/DD							
I	Near left superior temporal gyrus and left insula	-30	11	14	7.80	<.001	23461
		-38	-3	21	7.30		
		-40	-51	23	7.11		
J	Near right frontal lobe, right insula, and right medial frontal gyrus	31	6	15	8.87	<.001	26926
		29	13	11	8.64		
		32	-13	18	6.86		
TD/DD > FXS							
K	Near left frontal white matter (near superior frontal gyrus), left basal ganglia (caudate, putamen)	-22	18	11	6.78	<.001	9625
		-18	21	-4	6.66		
		-21	12	17	6.12		
L	Near left precentral gyrus and left postcentral gyrus	-31	-29	51	6.27	.007	4162
		-13	-17	58	5.43		
		-14	-3	58	4.68		
M	Near left precentral gyrus and inferior frontal gyrus	-43	-3	27	7.98	.001	5554
		-33	-13	44	4.46		
		-48	-16	34	4.25		
N	Near right medial frontal gyrus, right superior frontal gyrus, and right anterior cingulate gyrus	24	42	10	5.12	.003	4675
		17	59	5	5.07		
		19	42	-8	5.01		
O	Near right precentral gyrus and inferior frontal gyrus	47	-3	26	7.62	<.001	11828
		30	-30	50	6.42		
		34	-6	37	6.33		

Abbreviations: BA, Brodmann area; DD, idiopathic developmental delay; FXS, fragile X syndrome; iAUT, idiopathic autism; ROI, region of interest; TD, typically developing.

^aCapital letters denote ROIs depicted in eFigure 2C, eFigure 3C and D, and eFigure 4C and D.

total scores listed in Table 1. There were no significant correlations (Bonferroni corrected).

MULTIVARIATE PATTERN CLASSIFICATION

SVM Analysis

We used a linear SVM algorithm with a leave-1-out cross-validation procedure to examine how accurately the 4 participant groups could be distinguished based on spatial patterns of brain morphometry (**Figure 2**). Results using GM voxels only, WM voxels only, and GM and WM voxels combined were very similar and not significantly different from each other; therefore, the results from GM and WM voxels combined are reported here. Discriminability between FXS and iAUT was high using whole-brain SVM (accuracy, 90%). Maps derived from univariate and multivariate analyses showed similar patterns for

both approaches (Figure 2D). These results indicate that the brains of individuals with FXS and iAUT exhibit dissociable morphometric features in both GM and WM. Even when a subset of individuals with FXS who met criteria for AUT (FXS+A; eTable 2 for demographic characteristics) was compared with individuals with iAUT, the classification accuracy remained high with whole-brain SVM (82%), significantly greater than chance (*P* < .001), and not significantly different between the FXS (entire group) vs iAUT classification. High discrimination accuracy between FXS and iAUT was observed despite low and nonsignificant classification accuracy between these 2 groups using all available behavioral measures (47%; using RFE-SVM: 54%).

We also performed SVM classification using only brain regions that have been reported to show similar morphometric abnormalities in FXS and some studies of iAUT (ie, the caudate and cerebellar vermis²³). This analysis

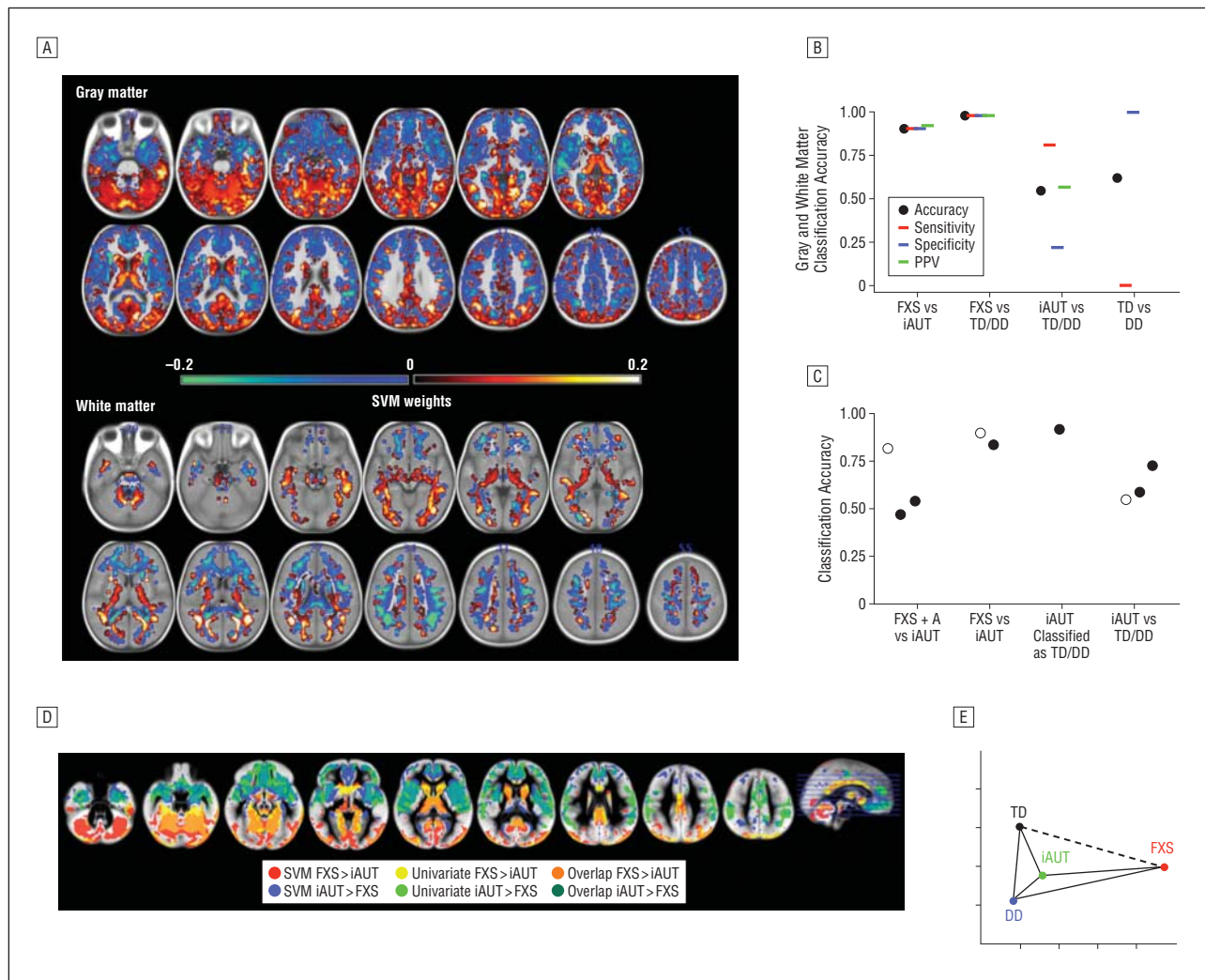


Figure 2. Pattern classification results. A, Whole-brain representation of pattern classification results from fragile X syndrome (FXS) vs idiopathic autism (iAUT) using gray matter and white matter voxels. Warm colors represent voxels with positive weight for the classification of FXS vs iAUT (FXS > iAUT) and cool colors represent negative weights (iAUT > FXS). The left side shows the right hemisphere. SVM indicates support vector machine. B, The SVM between-group classification accuracy using a combination of all gray matter and white matter as features. PPV indicates positive predictive value; TD, typically developing controls; and DD, idiopathic developmentally delayed controls. C, The SVM classification accuracy for various control analyses. For individuals with FXS who have a diagnosis of autism (FXS + A) vs those with iAUT, we show accuracy for the whole brain with dimensionality reduction using principal components analysis (open circle), all behavior (first solid circle), and behavior using recursive feature elimination (second solid circle). For FXS vs iAUT, we show accuracy for the whole brain with dimensionality reduction using principal components analysis (open circle) and using only the caudate and cerebellar vermis as features (solid circle). For iAUT classified as TD/DD using the classifier from FXS vs TD/DD, we show accuracy for the whole brain with dimensionality reduction using principal components analysis (solid circle). For iAUT vs TD/DD, we show accuracy for the whole brain with dimensionality reduction using principal components analysis (open circle), for only those areas significant in univariate voxel-based morphometric analyses (first solid circle), and for the whole brain using recursive feature elimination (second solid circle). D, Overlay of univariate and SVM analyses from the FXS vs iAUT contrast for gray matter (SVM weights thresholded based on $P = .05$, permutation-based correction). E, Brain-based representations of the 4 groups (TD, DD, FXS, and iAUT) using a self-organizing map. Solid lines indicate a euclidian distance greater than 1.

should maximize similarities between FXS and iAUT, thereby minimizing the ability to distinguish between the 2 groups. However, even using this subset of brain regions, classification accuracy between FXS and iAUT remained quite high (84%) and was not significantly different from the FXS vs TD/DD classification (87%; note that classification accuracy was 98% between individuals with FXS and TD/DD controls when the whole brain was used; also reported by Hoeft et al⁷). In contrast, classification accuracy between the 2 control groups (TD vs DD) was low (62% accuracy using the whole brain).

Further, when the classifier (model) derived from the FXS vs TD/DD classification was applied to iAUT, 92%

of the children were classified as TD/DD controls, suggesting that the brain regions that best distinguish FXS from TD/DD can also be used to reliably distinguish FXS from iAUT. In other words, these multivariate analysis techniques demonstrate that, as compared with controls, young boys with FXS represent a more unique and homogeneous group with respect to neuroanatomy than do boys with iAUT.

Classification accuracy discriminating iAUT from the TD/DD group using whole-brain SVM was 55% (not significantly greater than chance). Even when restricting the voxels to those that were significant from univariate analysis, classification analysis was 59% (not significantly greater than chance). When RFE-SVM was performed,

classification accuracy between iAUT and TD/DD improved from 55% to 73% ($P < .001$). However, this accuracy was still significantly lower than that derived from the FXS vs iAUT or FXS vs TD/DD classification analyses ($P < .001$). This finding implies that the joint information carried by a small number of voxels ($20\,224\text{ mm}^3$) rather than information from the whole brain can discriminate iAUT vs controls (although in this case, the performance of the classifier is less accurate than that derived for FXS vs controls).

Self-organizing Maps Analysis

To further visualize the relationship between the discriminative patterns characterizing the 4 groups (TD, DD, iAUT, and FXS), we used a technique known as self-organizing maps. This converts complex relationships between high-dimensional items into simple geometric relationships, adopting the method used by Formisano et al²¹ (Figure 2E). This brain-based representation also demonstrates the relative neuroanatomical resemblance (proximity) of iAUT to TD and DD as compared with FXS.

COMMENT

We examined neuroanatomical profiles of boys between the ages of 1 and 4 years who were diagnosed with iAUT and FXS, 2 neurodevelopmental disorders that at the descriptive level have overlapping behavioral phenotypes. However, iAUT is an etiologically heterogeneous and behaviorally defined neurodevelopmental disorder that involves deficits in social interaction and communication as well as rigid and repetitive patterns of behavior. On the other hand, FXS is a specific, genetically defined disorder caused by the silencing of the fragile X mental retardation 1 (*FMR1*) gene.²³ Many of the traits observed in those with FXS overlap with symptoms of iAUT, such as poor social interaction, qualitative abnormalities of communication, and stereotyped behavior; researchers have estimated that AUT spectrum disorders (ASDs) can be diagnosed in as many as 60% of those with FXS.^{23,25} The overlap in behavioral/cognitive symptoms reported in some studies has motivated some researchers to suggest overlapping neurobiological mechanisms underlying these 2 disorders.^{23,25} Indeed, prior research has suggested that there may be similar morphometric brain abnormalities in the caudate, in the posterior vermis of the cerebellum,²³ and in the connectivity between frontal and anterior temporal regions and their long-distance reciprocal and parietal connections.⁵

In this study, we show novel evidence that voxel-by-voxel brain volumes of boys with FXS and iAUT are on opposite extremes relative to controls for some GM and WM regions. Further, we demonstrate that morphometric spatial patterns are significantly different between FXS (and FXS+A) and iAUT, even at this very young age, using both univariate analysis as well as supervised and unsupervised machine learning methods. These distinct neuroanatomical patterns are present even though MVPA using diagnostic-behavioral data could not differentiate between FXS+A and iAUT. Another recent study²⁶ also

found neuroanatomical differences between AUT and FXS+A even though the 2 groups were behaviorally indistinguishable. Specifically, the group with AUT was found to have thinner cortex in the left anterior cingulate cortex and bilateral mPFC as compared with the group with FXS+A.

Several frontal and temporal GM and WM regions, including the mPFC, orbitofrontal cortex, STS, and TP as well as subcortical structures such as the amygdala, insula, and dorsal cingulum, showed patterns of volumetric differences that were on the opposite extremes for FXS (and FXS+A) and iAUT relative to controls such that $iAUT > controls > FXS$ (Figure 1C). This is somewhat different from the findings of our previous regions-of-interest-based volumetric study in the same population, in which we found greater amygdala volume in iAUT relative to both controls and FXS but no difference between controls and FXS.³ In this previous study, we also found that caudate volume was increased in both FXS and iAUT compared with controls. Another study that examined VBM of GM and conjunction analysis found regions where iAUT (or FXS) volumes were significantly different from those in both adults with FXS (or iAUT) and control adults.⁴ Thus, no previous studies have observed brain regions that show a pattern in which FXS and iAUT lie on opposite extremes relative to controls. This new finding is quite interesting as it suggests that these 2 disorders are neuroanatomically 2 sides of the same coin³ for some brain regions.

Using MVPAs of GM and WM, our results show that at least at this young age, the abnormal spatial patterns found in iAUT and FXS (and FXS+A) are strikingly distinct from one another. This was true even when we considered brain regions (caudate and cerebellar vermis) that have been proposed to be similarly aberrant for both disorders and when we considered only FXS+A. It is interesting that despite the robust classification power of MVPA for neuroanatomical data, FXS+A could not be distinguished from iAUT using multivariate approaches of behavioral data. Those with FXS (and FXS+A) exhibited much more obvious brain differences from our control groups than did those with iAUT. This was evidenced by significantly stronger classification accuracy between FXS (and FXS+A) and controls compared with iAUT and controls and by relatively weaker statistical difference between iAUT and controls as compared with FXS and controls.

While univariate analysis revealed several brain regions that were significantly different between iAUT and controls, our whole-brain SVM could not reliably differentiate between iAUT and controls. However, even when the SVM was restricted to voxels or features that showed significant effects in univariate analysis, classification accuracy remained relatively low. These results suggest that morphometric patterns have very little discriminative power between iAUT and controls.

It is possible that particular neuroanatomical differences shared by FXS and iAUT are related to specific aberrant behaviors exhibited by both of these groups. For example, in adults with ASD, neuroimaging data indicate that particular brain regions including the mPFC, temporoparietal junction, STS, and TP may be linked to

deficits in social cognition.^{11,27,28} The frontoinsula cortex (right > left) and anterior cingulate cortex are thought to be involved in intuitive judgments required by complex situations such as social interactions and have been suggested to play a critical role in ASD.²⁹ The caudate and cerebellar vermis, on the other hand, may be correlated with repetitive behavior symptoms.³⁰⁻³² Supplementary correlation analyses with social, communication, language, and repetitive behavior and regional GMV and WMV identified from univariate analyses did not show significant correlations in our sample of FXS or iAUT. Just as multivariate analyses such as SVM may be more accurate group classifiers, future studies using multivariate regression analyses to detect brain-behavior associations such as LASSO and support vector regression³³ may find these techniques to be more sensitive to the morphometric patterns that characterize specific behavioral phenotypes.

Interestingly, our results revealed that frontal and temporal regions implicated in social cognition, specifically the mPFC or anterior cingulate cortex, frontoinsula cortex, STS, TP, and amygdala, do show divergent patterns of abnormality in iAUT vs the patterns observed in our groups of FXS or FXS+A; that is, these social processing regions are significantly larger in iAUT and are smaller in FXS when compared with TD/DD controls (Figure 1). This dissociation was also observed in the dorsal frontoparietal WM tracts, which is interesting in light of the developmental disconnection hypothesis of AUT.⁵ These findings may partly explain recent evidence suggesting that the profile of social and communicative symptoms in FXS and iAUT are different² and do not support the hypothesis that overlapping neurobiological mechanisms underlie these 2 disorders. While beyond the scope of the current article, the dynamic nature of classification systems for AUT over time (eg, that described by Daniels et al³⁴) may also be a confounding factor in comparing iAUT with other developmental disorders such as FXS.

While the results in the current study were quite striking, there are important limitations that should be addressed in future investigations. For example, measures such as the ADI and ADOS are optimized to identify individuals with iAUT and may not be optimal to use in specific, more homogeneous populations such as individuals with FXS. Further, examination of more specific behavioral phenotypes such as social cognition may be more fruitful in pursuing this line of research. Finally, additional studies are needed to compare and contrast the trajectories of cognitive and behavioral development in children with FXS and iAUT. Such studies should relate these trajectories to profiles of neuroanatomical development to better model brain-behavior relationships associated with age at onset of symptoms, occurrence of developmental regression, and social developmental milestones.

The results of the current study, generated with both univariate VBM and multivariate SVM techniques, suggest that iAUT and FXS exhibit distinct neuroanatomical profiles relative to one another. Our results also indicate that iAUT is more likely to exhibit patterns similar to controls, likely owing to the neurobiological heterogeneity of these groups. That is, individuals are defined as having TD,

DD, or iAUT based on behavioral measures, whereas a diagnosis of FXS is established via a specific genetic difference shared by all members of the FXS group. It has been suggested that various ASD-associated genetic syndromes such as FXS, Angelman syndrome, and Rett syndrome may converge on common biological pathways or brain circuits that give rise to ASD.³⁵ However, our analyses of high-resolution imaging data from male toddlers with FXS and iAUT showed striking differences in brain morphometry at a very early age, even though we restricted our sample to males only and repeated our analyses using a subset of participants with FXS who met the behavioral criteria for AUT (FXS+A) to increase phenotypic similarity between our FXS and iAUT groups. It may be useful in the future to contrast individuals with homogeneous genetic conditions with and without ASD-like behavioral features (eg, FXS+A vs FXS-A). Although significant differences were not found between FXS+A and FXS-A in the present study (except for autistic symptoms), significant differences may be found within other ASD-associated genetic disorders.

On a related note, it may also be interesting to examine the detailed genetic, cognitive, and environmental profiles of children with FXS (or FXS+A) who were misclassified as having iAUT (or vice versa) based on structural magnetic resonance imaging, a quantitative endophenotype (eTable 3). The 12 individuals who were misclassified in our data set did not exhibit any notable demographic or behavioral characteristics that distinguished them from other individuals with FXS using univariate analysis, and no misclassified individuals with FXS had a diagnosis of AUT (ie, no misclassified individuals with FXS had FXS+A). Nonetheless, with a larger sample and detailed multivariate analyses of demographic characteristics, behavioral characteristics, genetic makeup, and behavioral changes over time, this route may provide invaluable information for future targets of iAUT research.

We demonstrate that FXS and iAUT are expressed as differing morphometric brain patterns. Further, this study has yielded intriguing evidence of the early brain phenotype in FXS. Our data may provide important clues regarding the altered neurodevelopmental pathways created by chronic diminished expression of the *FMR1* gene from a very early age. This work is particularly important for allowing researchers to establish a specific disease template in young humans in a manner comparable to research being performed in animal models of this disease (eg, fly, mouse). The creation of an early and accurate human brain phenotype for FXS in humans will significantly improve our capability to detect whether new disease-specific treatments can significantly alter the FXS phenotype in affected individuals.

Submitted for Publication: February 24, 2010; final revision received September 3, 2010; accepted September 12, 2010.

Published Online: November 1, 2010. doi:10.1001/archgenpsychiatry.2010.153

Correspondence: Allan L. Reiss, MD, Center for Interdisciplinary Brain Sciences Research, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, 401 Quarry Rd, Stanford, CA 94305-5795 (reiss@stanford.edu).

Author Contributions: Drs Hoeft and Walter contributed equally to this work.

Financial Disclosure: Dr Reiss is a consultant for Novartis, but this work was conducted and the manuscript written prior to his engagement as a consultant.

Funding/Support: This study was supported by grants R01MH050047 (Dr Reiss), T32MH019908 (Drs Reiss and Walter), R01MH064708 (Dr Reiss), R01MH064580 (Dr Piven), and MH061696 (Dr Piven) from the National Institute of Mental Health, grant P30HD003110 from the National Institute of Child Health and Human Development (Dr Piven), and the Canel Family Fund. Dr Hoeft was supported by the NARSAD Young Investigator Award, the Stanford Child Health Spectrum, and grant HD054720 from the National Institute of Child Health and Human Development. Ms Chang was supported by grant F31-AG032168 from the National Institutes of Health.

Online-Only Material: The eAppendix, eTables, and eFigures are available at <http://www.archgenpsychiatry.com>.

Additional Contributions: We sincerely thank all of the families and the Department of Radiology staff and faculty of the Lucile Packard Children's Hospital at Stanford who made the study possible. Chad Chappell, MA, Nancy Garrett, BA, Michael Graves, MChE, Cindy Hagan, BA, Cindy Johnston, MS, Arianna Martin, BA, Matthew Mosconi, PhD, Rachel Smith, BA, Cristiana Vattuone, BA, Christa Watson, BA, and Anh Weber, PhD, were involved in data collection, and Kelly Chen, BS, and Sweta Patnaik, MS, were involved in data processing.

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