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

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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.


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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. 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 postcentral, 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 AUT;2 (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 diagnostically 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.6-10

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 representative 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, childcare facilities, community media, and state-run agencies (eg, Regional Center system in California and Child Development Services 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 and quarterly newsletter, and through mailings to other regional FXS organizations. Children with iAUT and FXS were tested with the Autism Diagnostic Interview-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-
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-

### Table 1. Demographic Information

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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.

\(^a\)Pearson \( \chi^2 \) performed. No significant difference in any of the measures between sites (SU and UNC) for each diagnostic group.

\(^b\)Significant at \( P < .05 \).

\(^c\)Significant at \( P < .001 \).
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). 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, adjusted ADI sum (corrected for the number of items given to each child), ADOS composite score, ADOS severity score, and all ADI and ADOS subtests for FXS and iAUT separately.

MVP A 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). 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. 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. 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) 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. 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. To perform this limited voxel analysis, we coregistered bilateral caudate and vermis regions from the automated anatomical labeling atlas 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. 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 $2 \times 2$ two-dimensional map of 4 neurons (clusters). Prior to training, the number of features (voxels) was reduced using RFE-SVM; 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

Between FXS and iAUT (Table 1), the ADI and ADOS measures of social, communication, and repetitive behavior indicated greater behavioral problems in iAUT as
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).
**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, mediocingulate 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...
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 (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 vermis). This analysis...
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 analyses, classification accuracy 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 al.\(^\text{7}\) (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 \((\text{FMR1})\) gene.\(^\text{23}\) 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.\(^\text{23,22}\) The overlap in behavioral/cognitive symptoms reported in some studies has motivated some researchers to suggest overlapping neurobiological mechanisms underlying these 2 disorders.\(^\text{23,25}\) Indeed, prior research has suggested that there may be similar morphometric brain abnormalities in the caudate, in the posterior vermis of the cerebellum,\(^\text{2}\) and in the connectivity between frontal and anterior temporal regions and their long-distance reciprocal and parietal connections.\(^\text{3}\)

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 study\(^\text{26}\) 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.\(^\text{3}\) 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.\(^\text{4}\) 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 coin\(^\text{2}\) for some brain regions.

Using MVPA 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. The frontoinsular 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. The caudate and cerebellar vermis, on the other hand, may be correlated with repetitive behavior symptoms. 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 regression 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, frontoinsular 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. These findings may partly explain recent evidence suggesting that the profile of social and communicative symptoms in FXS and iAUT are different 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 al) 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. 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. 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.

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REFERENCES