ORIGINAL ARTICLE

Early Brain Overgrowth in Autism Associated With an Increase in Cortical Surface Area Before Age 2 Years

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Context: Brain enlargement has been observed in 2-year-old children with autism, but the underlying mechanisms are unknown.

Objective: To investigate early growth trajectories in brain volume and cortical thickness.

Design: Longitudinal magnetic resonance imaging study.

Setting: Academic medical centers.

Participants: Fifty-nine children with autism spectrum disorder (ASD) and 38 control children.

Intervention: Children were examined at approximately 2 years of age. Magnetic resonance imaging was repeated approximately 24 months later (when aged 4-5 years; 38 children with ASD; 21 controls).

Main Outcome Measures: Cerebral gray and white matter volumes and cortical thickness.

Results: We observed generalized cerebral cortical enlargement in individuals with ASD at both 2 and 4 to 5 years of age. Rate of cerebral cortical growth across multiple brain regions and tissue compartments in children with ASD was parallel to that seen in the controls, indicating that there was no increase in rate of cerebral cortical growth during this interval. No cerebellar differences were observed in children with ASD. After controlling for total brain volume, a disproportionate enlargement in temporal lobe white matter was observed in the ASD group. We found no significant differences in cortical thickness but observed an increase in an estimate of surface area in the ASD group compared with controls for all cortical regions measured (temporal, frontal, and parieto-occipital lobes).

Conclusions: Our longitudinal magnetic resonance imaging study found generalized cerebral cortical enlargement in children with ASD, with a disproportionate enlargement in temporal lobe white matter. There was no significant difference from controls in the rate of brain growth for this age interval, indicating that brain enlargement in ASD results from an increased rate of brain growth before age 2 years. The presence of increased cortical volume, but not cortical thickness, suggests that early brain enlargement may be associated with increased cortical surface area. Cortical surface area overgrowth in ASD may underlie brain enlargement and implicates a distinct set of pathogenic mechanisms.

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AUTISM IS A NEURODEVELOPmental disorder defined by the presence of social and communication deficits; stereotyped, repetitive behaviors; and a characteristic developmental course.[1] The presence of brain enlargement shown on magnetic resonance imaging (MRI) in autism is well established[2-5] and consistent with data showing enlarged head circumference[6-8] and increased brain weight.[9,10] Brain enlargement identified on MRI has been reported[11] in 51 two-year-old children with autistic disorder, the youngest cohort reported to date. These MRI data were consistent with retrospective, longitudinal head circumference data (from birth to age 3 years) that provide indirect evidence that increased brain growth may have its origins at the end of the first year of life.[11] Recently, a longitudinal study[12] of brain development replicated and extended the finding of generalized brain enlargement present by age 2 in children with autism.

The timing of brain enlargement in autism is of particular importance given new evidence from prospective behavioral studies[13] of infant siblings of individuals with autism showing typical social behaviors at 6 months followed by the onset of autistic social behavior at 12 months in infants who meet criteria for autism at 36 months. Results from these behavioral

Table 1. Sample Characteristics

Characteristic	Time 1		Time 2	
	ASD (n = 59)	Control (n = 38)	ASD (n = 36)	Control (n = 21)
Age, mean (SD), y	2.7 (0.32)	2.6 (0.52)	5.04 (0.41)	4.69 (0.46)
Male, No. (%)	51 (86)	28 (74)	32 (89)	15 (71)

Abbreviation: ASD, autism spectrum disorder.

studies suggest a period of typical development followed by the early postnatal onset of autistic disorder in the latter part of the first year or early second year of life. Direct evidence of the timing of early brain volume overgrowth in autism will focus future studies on this narrow window of brain development, providing important insights into potential underlying neural mechanisms and highlighting a potentially important period for early intervention and possible prevention.

The critical need for longitudinal brain imaging studies in conditions such as autism, characterized by clinical heterogeneity and the likelihood of nonlinear development, has been established by the seminal research of Giedd and colleagues.[14] We present herein a large longitudinal study using MRI to evaluate brain volume and cortical thickness (CT) changes in 2-year-old children with autism (the earliest date when valid diagnosis is considered possible) who were followed up at 4 to 5 years.

METHODS

SAMPLE

Participants in this longitudinal study included 59 children (aged 18-35 months) with autism spectrum disorder (ASD) and 38 comparison cases who underwent an initial behavioral assessment and brain MRI (time 1). Approximately 2 years later, when aged 4 to 5 years, this cohort of children underwent a second assessment and MRI (time 2). We attempted to have all children return 2 years later; however, this was not possible for some families, and some children were allowed to return up to 30 months later (when aged 5 years). There were no significant differences between the groups in the follow-up interval (ie, 3 months' difference between the ASD sample and controls). Thirty-eight children with ASD and 21 comparison cases were examined at the follow-up visit. The comparison group included typically developing (TYP) children 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), and the TYP group fell in the average range of functioning (estimated IQ, ≈100); therefore, lower-functioning children (ie, DD) were added to the control group. The DD control group was included to enrich the comparison sample for nonautistic children with low IQ. Autism is well known to include individuals with low IQ. The addition of nonautistic children with low IQ in the control group allowed us to take into account the association between IQ on brain volume.

Table 1 includes a description of the participants' characteristics. At time 1, there were 38 children in the comparison group (TYP, 26; DD, 12); at time 2, there were 21 (TYP, 15; DD, 6). Cases and controls did not differ significantly in age, and sex ratios were comparable in both groups. Mean (SD) age for the TYP group at time 1 was 2.49 (0.54) years and at time 2 was 4.59 (0.34); age for the DD group at time 1 was 2.83 (0.4) years and at time 2 was 4.97 (0.49). The TYP children were slightly younger than those in the other groups. There were no significant sex differences; at time 1, the TYP group was 77% male (20 boys) and the DD group was 67% male (8 boys).

A full description of the ascertainment and inclusion criteria is detailed in the report of Hazlett et al.[11] In brief, at study enrollment, medical records and developmental history were reviewed, and records were reevaluated at time 2. Children with ASD were referred after receiving a clinical diagnosis of autistic disorder. Children with DD were referred only if they had no known identifiable cause for their delay (eg, prematurity, genetic disorder, or neurologic disorder) and had no indication of a PDD. The TYP children were recruited from the community and were screened for ASD. All children with evidence of a medical condition thought to be associated with autism were excluded,[15] including fragile X syndrome, tuberous sclerosis, gross central nervous system injury (eg, cerebral palsy, significant complications of perinatal/postnatal trauma, or drug exposure), seizures, and significant motor or sensory impairments. Study approval was acquired from the University of North Carolina and Duke University institutional review boards, and written informed consent was obtained from parents or custodial guardians.

CLINICAL ASSESSMENT

At study entry, diagnosis for the children with autism was confirmed using the Autism Diagnostic Interview–Revised (ADI-R)[16] and the Autism Diagnostic Observation Schedule–Generic (ADOS-G).[17] Children were included in the ASD group only if they met ADI-R algorithm criteria for autism (all domains) and obtained ADOS-G scores consistent with autism. The same assessments were used at time 2 (aged 4-5 years), and all cases also met *DSM-IV* criteria[1] for autistic disorder. At the follow-up assessment, a small subset of children did not meet the original study criteria for autistic disorder (eg, ADI-R, ADOS-G, and *DSM-IV*) but continued to show evidence of symptoms consistent with a diagnosis of PDD–not otherwise specified. These children were classified as having PDD. The ASD sample therefore included 52 children with autism and 7 with PDD at time 1 and 33 children with autism and 5 with PDD at time 2. For our primary analyses, we included these children in the ASD group given that this approach has been used by many recent genetic studies of autism,[18] but we did examine them separately and have indicated comparisons where there are differences.

A largely identical battery of measures was administered at both time points, including the Mullen Scales of Early Learning,[19] Vineland Adaptive Behavior Scales,[20] Preschool Language Scale–4th edition,[21] behavioral rating scales, and a standardized neurodevelopmental examination, to exclude children with any notable dysmorphologic characteristics. At time 2, the Differential Abilities Scale was administered as an additional

Table 2. Cognitive Functioning and Adaptive Behavior of Sample

	Time 1		Time 2	
	ASD (n = 59)[a]	Control (n = 38)	ASD (n = 36)[a]	Control (n = 21)
Age, mean (SE), y	2.7 (0.32)	2.6 (0.52)	5.0 (0.41)	4.7 (0.46)
Male, No. (%)	51 (86)	28 (74)	32 (89)	15 (71)
Mullen, mean (SD)	54.32 (9.07)	90.74 (28.0)	58.97 (19.68)	97.25 (28.97)
Vineland, mean (SD)	61.04 (6.05)	87.84 (20.1)	52.78 (13.49)	83.05 (21.45)

Abbreviations: ASD, autism spectrum disorder; Mullen, Mullen Composite Standard Score; Vineland, Vineland Adaptive Behavior Composite Standard Score.
[a] Autism and pervasive developmental disorder combined.

cognitive measure.[22] All children in the autistic and DD groups received testing for fragile X syndrome (cytogenetic or molecular). Children with DD and TYP children were evaluated with the Childhood Autism Rating Scale[23] and excluded if they reached the cutoff score for autism (total score, ≥30). Medical records in the DD and TYP groups were also reviewed to exclude children with any possible evidence of ASD.

Table 2 presents the cognitive functioning and adaptive behavior characteristics of the sample. Many children with autism and DD failed to obtain a valid standard score on the Differential Abilities Scale at time 2, so we provide only estimates of cognitive functioning from their Mullen Scales of Early Learning. Cognitive functioning and adaptive behavior for children classified as having PDD were consistent with the autism sample, although the PDD group had slightly higher scores at time 2. At time 1, the mean (SD) IQ estimate was 56.1 (7.1) and at time 2 was 74.8 (32.3). Adaptive behavior for the PDD group at time 1 was 62.9 (6.4) and at time 2 was 65.5 (14.4). As noted in the "Methods" section, the lower-functioning children (DD) were added to the control group, and there were significant differences in IQ and adaptive behavior. At time 1, the TYP group had a mean estimated IQ of 107.0 (16.4) and adaptive behavior score of 98.4 (12.6). The children with DD had an estimated IQ of 55.5 (6.7) and adaptive behavior score of 65.8 (13.9). These group differences remained at time 2. The TYP group had a mean IQ of 113.9 (13.2) and adaptive behavior score of 95.1 (8.3). The DD group had a mean IQ of 58.5 (12.7) and adaptive behavior score of 55.0 (14.9).

MRI ACQUISITION

All MRI scans were performed at the Duke–University of North Carolina Brain Imaging and Analysis Center (1.5-T GE Signa MRI scanner; GE Healthcare, Fairfield, Connecticut). Image acquisition was designed to maximize gray/white tissue contrast for an 18- to 35-month-old child and included (1) a coronal T1 inversion recovery prepared: T1, 300 milliseconds; repetition time, 12 milliseconds; echo time, 5 milliseconds; 20° flip angle, at 1.5-mm thickness with 1 signal averaging, 20-cm field of view, and 256×192 matrix and (2) a coronal proton density/T2 2-dimensional dual fast spin echo: repetition time, 7200 milliseconds; echo time, 17/75 milliseconds; at 3.0-mm thickness with 1 signal averaging, 20-cm field of view, and 256×160 matrix. A localizer was included to monitor that the children were placed in the scanner in a systematic way. To evaluate scanner stability, we also collected geometric phantom data throughout the study. Core brain measures (intracranial volume, gray matter, and white matter) were graphed across the study and evaluated for any significant changes.

Imaging was performed in children with autism and those with DD with the use of moderate sedation (a combination of pentobarbital and fentanyl as per hospital sedation protocol) administered by a sedation nurse and under the supervision of a pediatric anesthesiologist in attendance. Monitoring of physiological function was conducted throughout the MRI and recovery. The TYP children underwent MRI without sedation. At age 2 years, MRI for all TYP children was performed in the evening while they were sleeping. At age 4 years, MRI was performed on 5 TYP children while awake after completing a behavioral training protocol to learn to lie still in the scanner. The remaining TYP children underwent MRI while sleeping for time 2. All MRIs were reviewed by a pediatric neuroradiologist and screened for significant abnormalities (eg, malformations or lesions).

IMAGE PROCESSING

The image processing procedures for these data are identical to those described in the initial report on this longitudinal study.[11] Briefly, images first underwent quality control checks to determine whether they were of sufficient quality to process. All images were rated by an experienced image processor who was blind to group membership. Each case was reviewed on a variety of criteria (eg, correct scan parameters used, motion artifact, and flow artifact) and assigned a rating based on image quality (1, poor; 2, mediocre; and 3, good). No images with poor quality ratings are included in this report.

The T1 and proton density/T2 images were then registered and aligned into a standardized plane along an anterior-posterior commissure axis.[11] The coregistered and aligned images were then processed for tissue segmentation using the Expectation Maximization Segmentation (EMS) image processing tool.[24,25] An "averaged" pediatric probabilistic brain atlas serves as a spatial prior and was automatically aligned to each child's brain using a linear affine transformation. The fully automatic EMS image processing pipeline includes multichannel registration, bias inhomogeneity correction, and nonbrain stripping in a single integrated tool. Gray, white, and cerebrospinal fluid tissue segmentations were produced for each child. Total brain volume (TBV) measures included total gray and white matter and all cerebrospinal fluid. Total tissue volume included all gray and white matter in the cerebrum (cerebral cortex), cerebellum, and brainstem.

Regional lobe volume measurements were obtained using a manually parcellated pediatric brain template (atlas) MRI used in the previous study,[11] which was then mapped onto each child's brain MRI using a fluid high-dimensional deformation algorithm described in that study. Delineated regions included the frontal, temporal, parietal, and occipital lobes; cerebellum; corpus callosum; interhemispheric fissure; 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 the cingulate was included with the frontal/parietal lobes. Cortical label maps were combined with the EMS tissue-classified images to pro-

duce gray/white/cerebrospinal fluid volumes for each of these lobe compartments.

Regional CT maps appropriate for pediatric MRI data were created by our group using ARCTIC (Automatic Regional Cortical Thickness) after attempts to use other available tools were unsuccessful. ARCTIC is a part of an image processing toolkit and is freely available (3D Slicer; http://www.nitrc.org/projects/arctic/). The computation uses the previous computed EMS tissue segmentation and lobar parcellation for a robust, image space–derived CT measurement. Measures were obtained in native (not stereotactic) space. To avoid extraction of topologically correct and precise cortical surfaces, which is challenging in pediatric brains, our CT analysis method used a discrete distance transform method that results in sparse sets of distance measurements between cortical surface and white matter boundaries along with detection of sulcal folds. The CT measurements were collected per lobe, and average values are reported herein as regional CT. We did not directly measure surface area (SA) but created an estimate of SA using a ratio term (SA=regional cortical volume [CV]/ regional CT). Regional CV was defined as the total cortical gray matter volume for the lobar region of interest. The lobar regions used to generate the CT and SA measures are identical to those defined for the lobe volumes and do not include subcortical structures.

STATISTICAL ANALYSES

A priori hypotheses were tested using general linear mixed models with repeated measures. In all models, brain volume was the dependent variable and diagnostic group (ASD, DD, and TYP), age, sex, and IQ were independent predictors. To account for the multiple regions of interest included in each model (eg, cerebrospinal fluid, gray matter tissue, and white matter tissue), a group of indicator variables was included that specified the region of interest for each observation.

Group was entered as a 3-level categorical variable. All group differences were calculated using the model-estimated coefficients. Comparisons with the controls used a weighted average of the 2 control groups (TYP + DD), which maximized the amount of variance that could be explained by group.

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 abilities for children who would otherwise score at the lower end of the standardized scale and be assigned values of less than 49. The IQ ratio was centered as the mean for all observations, and all main effects were estimated at these values unless otherwise specified.

For each group of analyses (total brain and lobe), 2 models were fitted to the data: the first included only group, age, sex, and IQ; the second model added TBV as a covariate to determine whether any brain volume differences were disproportionate to differences observed for TBV.

Tables 1 and 2 describe the sample characteristics. Age differences were observed (the TYP subgroup was slightly younger than the other groups), and age was included as a covariate. Sex was unequally distributed across groups and included as a covariate. The number of girls with autism was too small to perform separate analyses by group. The children with DD were included in the control group to control for IQ differences. Although IQ was not found to be a significant predictor between groups, comparisons were run both with and without IQ to be conservative. A difference in the retention rate for children with ASD (64%) vs controls (55%) was observed at time 2, but the study results were unchanged when those who did not return were dropped from the analyses. No significant differences in age, developmental IQ, adaptive behavior, sex, and symptom severity as reported on the ADI-R (for the ASD group only) were found between children who completed the study (2 time points) vs those who dropped out (1 time point).

Differences between the groups controlling for age, sex, and IQ were examined. Both groups showed increases in brain volume over time in all areas measured, and there was no significant difference in rate of brain growth between groups throughout the study. Because age × 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 a total volume (sides combined).

To assess regional CT, a linear mixed model similar to that used to assess volume differences was fitted and included up to 12 measures per child (3 lobes/side/time). This model was fitted to the unadjusted average CT for each lobe and hemisphere. Age, sex, and IQ were included as covariates in the models along with group and a set of indicator variables that delineated hemisphere and region. A second identical model was fitted to examine the estimate for SA.

RESULTS

Growth trajectories for TBV, total gray matter volume (TGV), and total white matter volume (TWV) are displayed in **Figure 1**. Trajectories of growth in cerebral cortical lobe regions (frontal, temporal, and parieto-occipital) appear in **Figure 2**. Both the ASD and control groups showed an increase in cerebral CV in all compartments of the brain; however, there was no significant difference in rate of brain growth between the groups at ages 2 and 4 to 5 years. Brain volume enlargement observed in individuals with ASD at 2 years continued to be present, to the same degree, at 4 to 5 years.

Mean group differences are reported in **Table 3** for ASD vs controls and in **Table 4** for autistic children vs the TYP and DD control subgroups. Those with ASD had significant enlargement in TBV, total tissue volume (TGV + TWV), TGV, and TWV, with a 9% enlargement of cerebral cortex volume compared with controls. Cerebellar volume did not differ significantly between the ASD and control groups. Children with ASD had enlargement in both gray and white matter volume for all cortical lobes, but, after controlling for TBV, only temporal lobe white matter volume remained significantly enlarged in comparison with controls. This same pattern of generalized volume enlargement in the ASD group for the cerebrum and cortical lobes was also seen in the TYP and DD subgroup comparisons (Table 4).

Differences for all regions and tissues remained significant after removal of the subset of the ASD group (n=7) who met criteria for autistic disorder at time 1 and PDD-not otherwise specified (but not autistic disorder) at time 2. Findings also remained the same after the removal of 2 controls observed to have the smallest TBV (Figure 1).

Average regional CT was measured in the frontal, temporal, and parieto-occipital lobe regions. Group raw means for CT by lobe region are provided in **Table 5**. We ex-

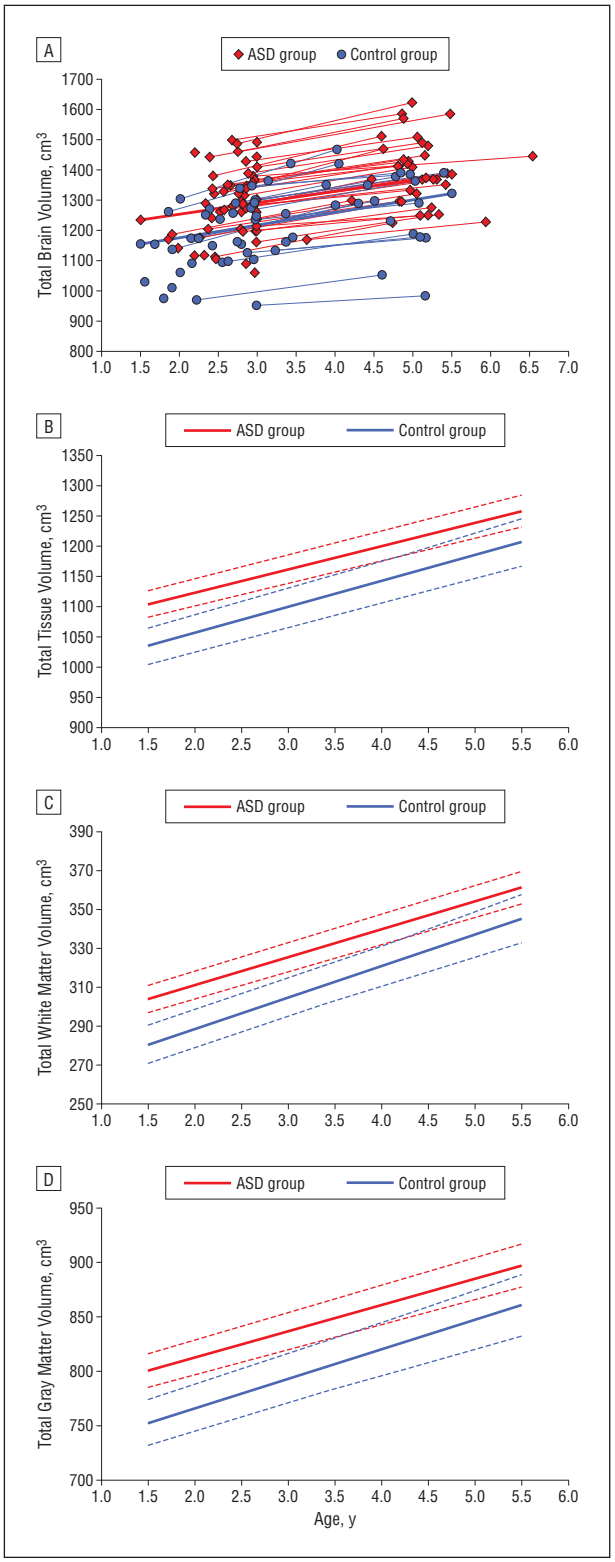

Figure 1. Trajectory of development: total brain volume, total tissue volume, total white matter volume, and total gray matter volume. Panels show the subject trajectories (scatterplot) for total brain volume (A) and mean group trajectories with 95% confidence interval bands (dashed lines) for total tissue volume (B), total white matter volume (C), and total gray matter volume (D). ASD indicates autism spectrum disorder.

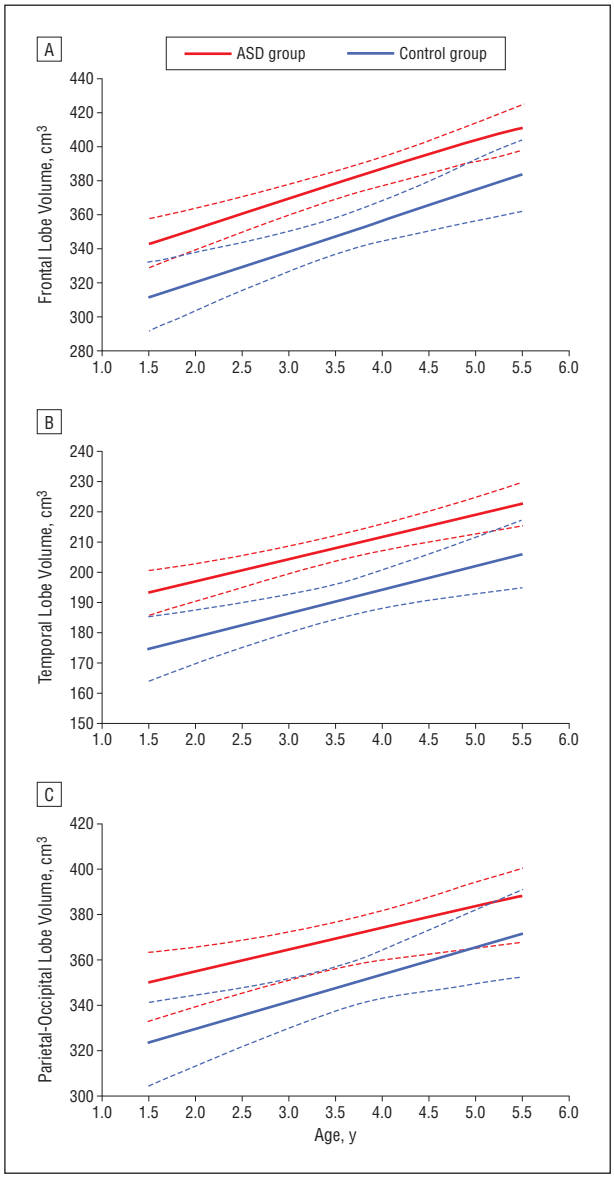

Figure 2. Trajectory of development: cerebral cortical lobe volumes. Panels show the mean group trajectories with 95% confidence interval bands (dashed lines) for the cortical lobe volumes. Cortical regions displayed include frontal (A), temporal (B), and parieto-occipital (C) lobes. Combined volumes (right + left hemisphere) for each lobar region are shown. ASD indicates autism spectrum disorder.

amined regional estimates of CT summarized over the cortical lobes. There was no significant interaction between group, age, and regional brain volume. We observed no significant group differences in CT for any of the lobar regions measured (frontal: $t=0.1$, $P=.92$; temporal: $t=-0.33$, $P=.74$; and parieto-occipital: $t=-0.05$, $P=.96$). We then created an estimate of SA using a ratio term (SA = regional CV/regional CT) to examine whether there were differences in CT after adjusting for CV. For this comparison, we found significantly increased estimates of SA in the ASD group compared with the control group for all 3 cortical regions measured (frontal: $t=3.79$, $P<.001$; temporal: $t=3.49$, $P<.001$; and parieto-occipital: $t=3.18$, $P=.002$). In summary, we found no significant differences in CT but did find increased estimates of SA in children with ASD compared with controls. Trajectories of change over time in our estimate of SA (CV/CT) appear in **Figure 3**.

Table 3. Adjusted Means for Brain Volumes and Group Comparisons for ASD vs Control Group[a]

Volume, cm³	ASD, Mean (SE)	Control, Mean (SE)	Comparisons, ASD vs Control[b]		
			Difference (SE)	*P* Value	Change, %
Total brain	1310.4 (13.0)	1238.8 (18.0)	71.6 (22.7)	.002	6
Total tissue	1181.8 (11.7)	1120.1 (16.2)	61.7 (20.4)	.003	6
Total gray matter	849.2 (8.4)	807.2 (11.7)	42.1 (14.6)	.005	5
Total white matter	332.6 (3.6)	312.9 (5.0)	19.6 (6.3)	.002	6
Cerebrospinal fluid	128.6 (2.5)	118.7 (3.4)	9.9 (4.4)	.03	8
Cerebellum	117.9 (1.3)	118.1 (1.8)	–0.2 (2.2)	.93	0
White matter	18.5 (0.3)	19.1 (0.4)	–0.7 (0.5)	.17	–3
Gray matter	99.4 (1.1)	99.0 (1.5)	0.5 (1.9)	.81	0
Cerebral cortex	986.0 (8.6)	908.3 (11.9)	77.7 (15.1)	<.001	9
White matter	280.9 (2.9)	253.3 (3.9)	27.6 (5.2)	<.001	11
Gray matter	705.1 (6.0)	655.0 (8.3)	50.1 (10.3)	<.001	8
Parieto-occipital lobe	369.2 (3.6)	347.2 (5.0)	21.9 (6.7)	.001	6
White matter	107.7 (1.2)	99.1 (1.6)	8.6 (2.1)	<.001	9
Gray matter	261.5 (2.6)	248.1 (3.5)	13.3 (4.7)	.005	5
Temporal lobe	207.9 (2.1)	190.3 (2.9)	17.6 (3.9)	<.001	9[c]
White matter	44.6 (0.6)	39.8 (0.8)	4.8 (1.0)	<.001	12[c]
Gray matter	163.3 (1.7)	150.6 (2.2)	12.8 (3.1)	<.001	8
Frontal lobe	377.5 (4.0)	347.5 (5.4)	30.0 (7.3)	<.001	9
White matter	114.9 (1.4)	104.1 (1.9)	10.8 (2.6)	<.001	10
Gray matter	262.6 (2.7)	243.4 (3.6)	19.2 (4.9)	<.001	8

Abbreviation: ASD, autism spectrum disorder.
[a] The adjusted means are the estimated adjusted means for total and lobar brain volumes at age 3.5 years (the approximate mean age for our sample).
[b] Controlling for age, sex, and IQ.
[c] Comparisons for brain volumes with enlargement (eg, cerebrum and cortical lobes) that remained significantly (*P* = .05) enlarged after controlling for total brain volume.

COMMENT

In this longitudinal MRI study of very early brain volume development in individuals with ASD, we observed generalized cerebral cortical enlargement in children with ASD at both 2 and 4 to 5 years of age. Rate of cerebral cortical growth across multiple brain regions and tissue compartments in children with ASD parallels that seen in controls, indicating that there is no increased rate of cerebral cortical growth during this age interval. Our findings provide evidence that increased brain volume at age 2, largely due to increased cerebral CV, results from an increased rate of brain growth occurring before 2 years of age. Together with previously reported findings from a longitudinal study of head circumference[11] and a recent longitudinal MRI study of early brain volume development,[12] these data provide further evidence that brain overgrowth in autism occurs in the early postnatal period. In the cross-sectional analysis of 2-year-old children,[11] those with autism had significantly enlarged gray and white matter volumes compared with the DD subgroup, but only white matter volumes were enlarged compared with the TYP subgroup. The longitudinal analyses reveal increased white and gray matter volume in autism vs both the TYP and DD control subgroups. Given the fact that no significant differences in our data were seen between autistic and control subjects at either age point in this longitudinal study, we feel confident in our conclusion that volume increases are evident in this sample of autistic subjects. Of course, the small size of the control subgroups compels us to be most certain about our findings with respect to the total sample of controls.

The findings presented herein point to increased cerebral cortical SA rather than increased CT as the underlying factor in the larger cerebral cortical gray matter volume observed in very young children with ASD. Emerging literature on cortical maturation in older males with ASD has found evidence for decreased CT in adolescence[26-28]; it may be that a period of cortical thinning occurs in ASD after childhood. It is unclear at this point whether increased white matter results in enlarged gray matter, SA, or both or whether a common etiologic source causes both increased white matter and SA. As we have learned from a study of the *MAOA* gene,[29] in which *MAOA* effects were found on both white and gray matter volumes but not with the serotonin transporter, the biological mechanisms underlying cortical growth are complex. Increased SA results from an increase in the number and/or size of cerebral cortical gyri. Several studies suggest that such gyral abnormalities may be present in individuals with ASD. Nordahl et al[30] observed "cortical folding abnormalities" in autism, and Lenroot et al[31] reported an increase in SA in 4- to 5-year old children with ASD. Kates et al[32] noted abnormal "gyrification" in monozygotic twins discordant for autism. Raznahan et al[33] reported that adults with ASD differ from controls in the relationship between a key genotype for determining regional CV (brain-derived neurotrophic factor or BDNF val66met) and CV and SA (but not CT). Petropoulos et al[34] reported prolonged T2 relaxation for cortical gray matter in a large sample of children aged 2 to 4 years with

Table 4. Adjusted Means for Brain Volumes for Subgroup Comparisons[a]

Volume, cm³	TYP, Mean (SE)	DD, Mean (SE)	Comparisons, ASD vs TYP[b]		Comparisons, ASD vs DD[b]	
			Difference (SE)	*P* Value	Difference (SE)	*P* Value
Total brain	1258.0 (21.2)	1219.6 (29.4)	52.4 (25.7)	.044	90.8 (32.1)	.006
Total tissue	1136.7 (19.1)	1103.5 (26.5)	45.1 (23.1)	.053	78.3 (28.9)	.008
Total gray matter	816.6 (13.8)	797.7 (19.0)	32.6 (16.7)	.053	51.5 (20.7)	.06
Total white matter	320.1 (5.9)	305.8 (8.1)	12.5 (7.2)	.09	26.8 (8.8)	.003
Cerebrospinal fluid	121.3 (4.5)	116.0 (5.4)	7.2 (5.6)	.20	12.6 (5.9)	.04
Cerebellum	120.6 (2.1)	115.7 (2.9)	–2.7 (2.6)	.30	2.3 (3.2)	.48
White matter	19.4 (0.5)	18.9 (0.5)	–0.9 (0.6)	.15	–0.4 (0.6)	.49
Gray matter	101.2 (1.8)	96.7 (2.5)	–1.8 (2.1)	.41	2.7 (2.7)	.33
Cerebral cortex	919.9 (14.8)	896.7 (19.0)	66.1 (17.7)	<.001[c]	89.3 (20.8)	<.001
White matter	256.3 (5.2)	250.3 (6.1)	24.6 (6.5)	<.001	30.6 (6.7)	<.001
Gray matter	663.6 (10.0)	646.4 (13.3)	41.5 (11.8)	<.001	58.7 (14.6)	<.001
Parieto-occipital lobe	350.9 (6.9)	343.6 (7.6)	18.3 (8.6)	.04	25.6 (8.4)	.003
White matter	100.3 (2.2)	97.9 (2.4)	7.4 (2.8)	.01	9.8 (2.6)	<.001
Gray matter	250.5 (4.8)	245.7 (5.4)	10.9 (6.0)	.07	15.8 (6.0)	.009
Temporal lobe	193.8 (4.2)	186.8 (4.3)	14.1 (5.3)	.008	21.1 (4.7)	<.001
White matter	39.8 (1.1)	39.8 (1.1)	4.8 (1.4)	<.001[c]	4.8 (1.3)	<.001
Gray matter	154.0 (3.3)	147.1 (3.3)	9.3 (4.1)	.02	16.3 (3.7)	<.001
Frontal lobe	354.0 (7.7)	341.0 (8.2)	23.5 (9.6)	.02	36.5 (9.0)	<.001
White matter	105.7 (2.7)	102.6 (2.9)	9.2 (3.5)	.009	12.3 (3.2)	<.001
Gray matter	248.3 (5.1)	238.4 (5.5)	14.3 (6.4)	.03	24.2 (6.1)	<.001

Abbreviations: ASD, autism spectrum disorder; DD, developmental delay; TYP, typically developing children.

[a] The adjusted means are the estimated adjusted means for total and lobar brain volumes at age 3.5 years (the approximate mean age for our sample) for children in the ASD group compared with TYP and DD children.

[b] Controlling for age, sex, and IQ.

[c] Comparisons for brain volumes with enlargement (eg, cerebrum and cortical lobes) that remained significantly ($P = .05$) enlarged after controlling for total brain volume.

ASD compared with TYP controls. Our findings and the observation by Petropoulos et al suggest that abnormal early development of gray matter is associated with ASD.

Human studies[29,35] have suggested several candidate genes that may play a role in the increased cerebral CV in ASD. The likely importance of epistasis in brain overgrowth in ASD is underscored by a mouse study[36] of deletions in the serotonin transporter and *PTEN* genes showing an interactive effect, increasing both brain volume and autisticlike behaviors in mice. Family studies[37] have revealed that both cortical SA and CT are highly heritable but unrelated genetically, suggesting distinct genetic architecture underlying these phenomena. The finding of SA but not CT differences provides a narrower phenotypic target for future studies exploring the genetic basis of autism, as distinct neurobiologic mechanisms are thought to underlie these 2 determinants of CV.[38,39]

Surface area is thought to be determined by division of progenitor cells in the embryologic periventricular area (with increased progenitor cells occurring in association with increased cortical SA), whereas CT is thought to reflect variation in dendritic development (arborization and pruning) in gray matter[40,41] or myelination.[42] Molecular studies[39] in mice have demonstrated the role of β-catenin in regulating cerebral cortical size (and resultant increases in cortical SA but not CT) by controlling the generation of neural precursors. Glycogen synthetase kinase 3 was recently shown to cause massive hyperproliferation of neural progenitor cells in mice, resulting in large brains with increased convolutions.[43] Glycogen synthetase kinase 3 interacts with the phosphatidyl inositol 3 kinase pathway, implicated in several neurodevelopmental disorders (eg, fragile X syndrome and tuberous sclerosis) that are characterized by autistic behavior.[43,44] Glycogen synthetase kinase 3 also interacts with the receptor tyrosine kinase signaling system, which has been linked to idiopathic autism.[45] These various pathways for brain overgrowth clearly point to areas that need further study in autism.

Retrospective head circumference data on a large sample of children with ASD compared with local controls from birth to age 3 years suggested that increased head size in ASD has its onset around 12 months of age.[11] We hypothesize that this increased head size was the result of increased brain size and that brain overgrowth had its onset in the latter part of the first year of life. Longitudinal behavioral studies of infants at high genetic risk for ASD, who are later diagnosed with ASD at 36 months, report no difference in social behavior at 6 months in comparison with controls, whereas marked deficits in reciprocal social interaction are observed by 12 to 14 months.[13,46] These behavioral studies suggest that the onset of autistic behavior has its origins in the latter part of the first year of life. The temporal relationship between the onset of both autistic behavior and brain overgrowth at the end of the first year of life suggests a relationship between these 2 phenomena, specifically, that increased rate of brain growth may be linked to the onset of autistic symptoms.

It is possible that brain overgrowth directly results in the development of autistic behavior, perhaps through

Table 5. Raw Means for Regional Cortical Thickness for ASD and Controls

Group and Region	Side	Cortical Thickness, Mean (SD), cm²	
		Time 1	Time 2
ASD		(n = 54)	(n = 35)
Frontal lobe	L	4.36 (0.15)	4.32 (0.15)
	R	4.45 (0.20)	4.38 (0.14)
Temporal lobe	L	3.92 (0.21)	3.86 (0.21)
	R	3.93 (0.17)	3.90 (0.17)
Parieto-occipital lobe	L	3.68 (0.24)	3.64 (0.25)
	R	3.57 (0.19)	3.60 (0.15)
Control		(n = 36)	(n = 18)
Frontal lobe	L	4.36 (0.16)	4.32 (0.15)
	R	4.41 (0.20)	4.34 (0.14)
Temporal lobe	L	3.92 (0.18)	3.91 (0.24)
	R	3.90 (0.18)	3.95 (0.19)
Parieto-occipital lobe	L	3.62 (0.15)	3.65 (0.17)
	R	3.57 (0.15)	3.58 (0.19)

Abbreviations: ASD, autism spectrum disorder; L, left; R, right.

a physical disruption of neural circuitry. An alternative hypothesis is that brain overgrowth is a secondary response to a more proximal event that affects downstream remodeling of neuronal processes. Disruption in experience-dependent cortical refinements caused by impaired synaptic plasticity has been reported[47] in a mouse model of Angelman syndrome, a disorder thought to be associated with autistic behavior. Similarly, disruptions in normal synaptic plasticity and experience-dependent neuronal development have been observed in a mouse model of fragile X syndrome,[48] a disorder also associated with autism. Consistent with the idea that autism is linked to impaired experience-dependent cortical development, a recent study[49] in a sample of autistic individuals observed a high number of diverse mutations known to cause defective expression of activity-driven genes. Alterations in synapse development have also been proposed as a common mechanism in a number of neurodevelopmental disorders, including autism.[50]

A potential limitation of the study reported here stems from our inability to measure SA directly in very young children. As such, we were able to obtain only regional estimates of CT and an estimate of SA, and the SA findings should therefore be considered preliminary. Although mean CT in each lobar region is not necessarily indicative of uniformity of CT throughout the cerebral cortical lobes (there exists normal variation in CT, known to be increased, for example, in heteromodal association areas[51]), the convergence of CT findings across the 3 cortical regions measured supports the validity of our findings. Software to enable local CT and SA measurement in the developing pediatric brain is currently under development in our laboratory and will provide an important step in characterizing early brain volume changes in individuals with ASD. An additional potential limitation of our study was the use of sedation with some participants (ASD, DD) and not others (TYP). However, we have no reason to believe that sedation at the time of MRI had any significant effect

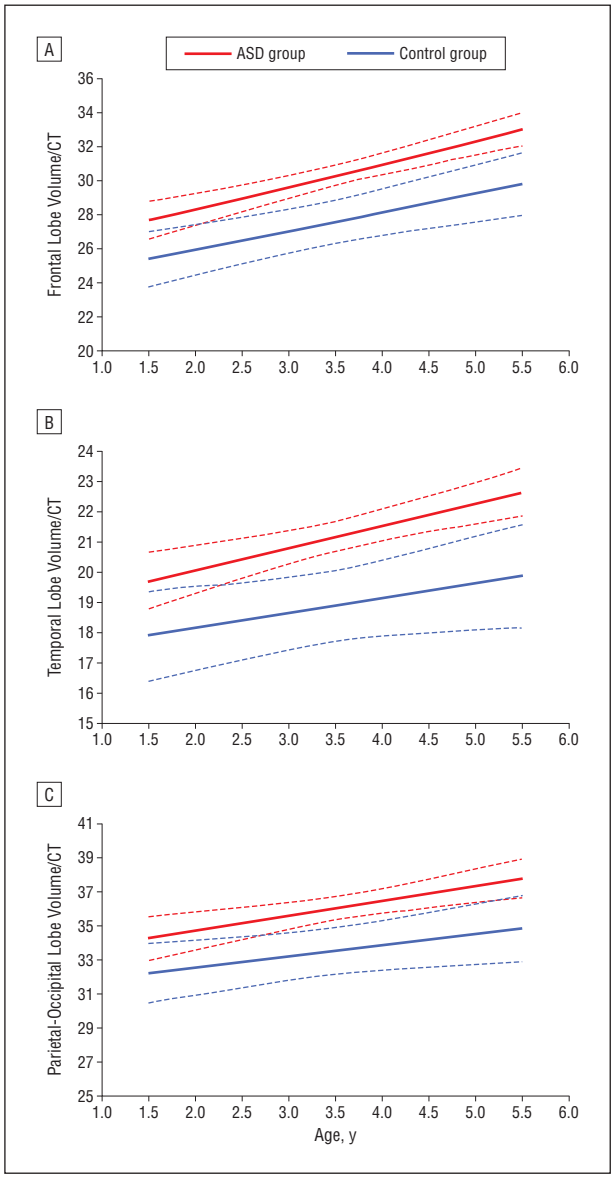

Figure 3. Trajectory of development: surface area. Panels show the mean group trajectories with 95% confidence interval bands for an estimate of surface area (surface area = regional cortical volume/regional cortical thickness [CT]) in the frontal (A), temporal (B), and parieto-occipital (C) lobes. The measure of cortical volume in this case refers only to gray matter volume. ASD indicates autism spectrum disorder.

on CV; to our knowledge, there is no evidence in the literature to suggest a state effect that would confound our results.

Studies under way by our group (http://www.ibis-network.org/) are prospectively (at 6, 12, and 24 months) examining MRI/diffusion tensor imaging brain and behavior development in infants at high risk for ASD, further characterizing the timing of brain-behavior changes in this disorder. Given the findings in other brain disorders (eg, Parkinson, Alzheimer, and Huntington diseases), in which brain changes are well known to precede the cognitive and/or behavioral manifestation of symptoms,[52-54] observations from the present study support future research aimed at identifying early (younger than 2 years) brain markers that may increase predic-

tion of ASD risk (eg, maturational differences in selected diffusion tensor imaging fiber tracts in infants with high genetic risk for ASD). Studies should continue the strategy of longitudinal imaging to more definitively characterize the pattern of brain changes as individuals with ASD age across the lifespan.

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