# A Preliminary Longitudinal Magnetic Resonance Imaging Study of Brain Volume and Cortical Thickness in Autism

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**Background:** Autism is a developmental neurobiologic disorder associated with structural and functional abnormalities in several brain regions including the cerebral cortex. This longitudinal study examined developmental changes in brain volume and cortical thickness (CT) using magnetic resonance imaging (MRI) in children with autism.

**Methods:** MRI scans and behavioral measures were obtained at baseline and after a 30-month interval in a sample of male subjects with autism ($n = 18$) and healthy age-, and sex-matched control subjects ($n = 16$) between ages 8 and 12 years at baseline.

**Results:** No differences in brain volumes were observed between the autism and control subjects at baseline or follow-up. However, differences in total gray matter volumes were observed over time with significantly greater decreases in the autism group compared with control subjects. Differences in CT were observed over time with greater decreases in the autism group compared with control subjects in several brain regions including the frontal lobe. When accounting for multiple comparisons, differences between the two groups became nonsignificant except for changes in occipital CT. Furthermore, associations were observed between several clinical features and changes in CT with greater thinning of the cortex being correlated with more severe symptomatology.

**Conclusions:** Findings from this study provide preliminary evidence for age-related changes in gray matter volume and CT in children with autism that are associated with symptoms severity. Future longitudinal studies of larger sample sizes are needed to evaluate developmental changes and examine the relationships between structural abnormalities and clinical expressions of the disorder.

**Key Words:** Cerebral cortex, clinical features, development, repetitive behaviors, severity, social deficits

Autism is a complex neurodevelopmental disorder associated with structural and functional abnormalities in several brain regions including the cerebral cortex. Neuroimaging studies have reported a wide range of anatomic alterations, but increased brain volume in young children with autism is one of the most consistent neurobiologic findings (1–6). However, it remains unclear how long this enlargement persists and whether increased volume comprises primarily gray or white matter. In one of the first studies to observe age-related changes, an increase in brain size was observed in children and adolescents with autism but not in adults (2). A larger study of children with autism (1) reported that by age 2–4 years, 90% of autistic boys had a brain volume larger than normal matched control subjects, whereas older autistic children and adolescents did not exhibit this enlargement. Young children had more cerebral (18%) and cerebellar (39%) white matter and more cerebral cortical gray matter (12%) when compared with control subjects. While examining the same sample, Carper *et al.* (3) observed increased size of all cortical lobes with the frontal showing the greatest enlargement. Interestingly, developmental changes were also observed in frontal, temporal, and parietal white matter with frontal and temporal gray matter volume showing a slower increase in size in the autistic group compared with control subjects. These observations suggest the existence of age-related changes in brain size in autism and longitudinal studies are warranted to shed light on this process.

Although less robust than the finding of enlarged cerebral volume, several investigations have examined the size of the corpus callosum in autism and revealed a reduction in one or more components of this primary white matter tract, including the anterior (genu and rostrum), middle (body), or posterior (isthmus and splenium) subregions (7). This observation appears to be consistent with the theory of aberrant connectivity in autism. In fact, several investigations have reported lower functional connectivity in individuals with autism compared with control subjects while performing a sentence comprehension task (8), a visuomotor task (9), and an inhibition task (10). Although mounting evidence is emerging to support this hypothesis, the exact structural underpinnings of these disturbances remains to be determined. White matter disturbances and brain surface parameters such as cortical gray matter thickness and gyrification patterns have been implicated in the pathophysiology of the aberrant connectivity syndrome.

Over the past decade, investigations of cortical white matter have reported structural alterations in autism. Volumetric and diffusion tensor imaging (DTI) studies have examined several brain regions and observed diffuse abnormalities. Volumetric differences in white matter regions have been found in young children with autism (11), and these alterations appear to affect mostly outer radiate compartments (12). In addition, DTI studies have found reduced fractional anisotropy (a measure of coherent fiber tract directionality) in white matter regions including the anterior cingulate, the temporoparietal junctions, the corpus callosum, ventromedial prefrontal cortices, and the temporal lobes (13,14). Although these findings implicate white matter tract abnormalities in autism, it is unlikely that such findings exist in the absence of cortical gray matter abnormalities. Abnormally

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narrow cortical minicolumn architecture has been a well-documented finding in autism, and it is hypothesized that such abnormal architecture could result in fewer long-distance connecting fibers compared with abundant short-distance connections (15).

Surface parameters including cortical thickness (CT) have been examined in autism (16,17). Postmortem studies have reported alterations of cortical gray matter including increased CT and neuronal density (16). Abnormalities in CT have also been reported in neuroimaging studies. Increased total cerebral sulcal and gyral thickness were observed in a sample of 17 male children with autism compared with 14 healthy sex- and IQ-matched control subjects (17). This increase was observed primarily in the temporal and parietal lobes but not in the frontal and occipital. These preliminary findings indicate that increased CT may contribute to the increased gray matter volume and total brain size that have been observed in children with autism and may also be related to anomalies in cortical connectivity. However, findings of increased CT have not been consistently reported, with recent reports describing a decrease (18) or even the absence of any abnormalities in CT in autism (19). These reports have examined primarily adults with autism, whereas Hardan *et al.* (17) included only children. Therefore, and in light of the developmental abnormalities observed in autism, it is possible that increased CT is present in children but not in adolescents, and longitudinal studies are warranted to examine these age-related changes.

The goal of this investigation was to conduct a longitudinal study to examine total brain volume and CT in a sample of male children with autism who were evaluated at a 30-month interval. On the basis of available evidence, we hypothesized that increased total brain volume and CT would be observed at baseline but not at follow-up. We also predicted that structural changes observed over time would be associated with clinical features.

## Methods and Materials

### Participants

Thirty-four children and adolescents (autism, $n = 18$; control, $n = 16$) were selected from an original sample of 40 who met inclusion and exclusion criteria. One individual with autism was not willing to be part of the procedures at follow-up because of the discomfort he experienced during the magnetic resonance imaging (MRI) scanning. Three individuals with autism were excluded because they did not have good quality scans at either baseline or follow-up. Two control subjects were not able to participate in the follow-up MRI scanning because the family had moved to a different state. The study was confined to boys because the sample size was too small to accommodate structural variability associated with sex. The institutional review board approved the methodology of the study, including MRI scanning of minors.

Subjects were 18 boys with autism and 16 healthy male control subjects aged between 8 and 12 years at baseline. Data from a subsample of this group that included 17 children with autism and 14 healthy comparison subjects has previously been published (17).

Participants with autism represented all consecutive referrals to a research clinic eligible to participate in the study. The diagnosis of autism was established through expert clinical evaluation and two structured research diagnostic instruments, the Autism Diagnostic Interview—revised (ADI-R) (20) and the Autism Diagnostic Observation Schedule (ADOS) (21). Children with secondary autism such as tuberous sclerosis and fragile X were excluded.

**Table 1.** Characteristics of Individuals with Autism and Control Subjects at Baseline and Follow-Up

| | Autism $n = 18$ | | Control $n = 16$ | | *t* Test *df* 32 | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | *t* | *p* |
| Time 1 | | | | | | |
| Age (Years) | 10.9 | 1.2 | 10.7 | 1.2 | .613 | .544 |
| FSIQ | 93.9 | 17.0 | 113.1 | 12.9 | −3.668 | .001 |
| SES | 4.5 | .6 | 4.3 | .6 | 1.214 | .234 |
| ADI-R | 51.2 | 7.4 | — | — | — | — |
| ADOS | 15.5 | 3.1 | — | — | — | — |
| Time 2 | | | | | | |
| Age (Years) | 13.3 | 1.4 | 12.6 | 1.2 | 1.428 | .163 |

ADI-R, Autism Diagnostic Interview—Revised; ADOS, Autism Diagnostic Observation Schedule; FSIQ, full-scale IQ; SES, socioeconomic status.

Control subjects were children recruited from the community through advertisements in areas socioeconomically comparable to those of the families of origin of the autistic subjects. Socioeconomic status for all subjects was assessed using the Hollingshead method (22). Control subjects were screened with face-to-face interviews, questionnaires, telephone interviews, and observation during psychometric tests, and individuals with a family history of any neuropsychiatric disorder, such as autism, learning disability, affective disorders, and schizophrenia, were not included. Potential subjects with a history of birth asphyxia, head injury, or a seizure disorder were also excluded. All control subjects had a full-scale IQ (FSIQ) > 70, and their academic skills were assessed using the Wide Range Achievement Test—Revised (23). Potential subjects were excluded if found to have evidence of birth asphyxia, head injury, or a seizure disorder. Exclusions were based on neurologic history and physical examination, as well as laboratory testing when indicated. The Wechsler Intelligence Scale for Children—III was administered to measure IQ. Participant demographics are provided in Table 1.

### MRI Scans

Brain scans were obtained using the same acquisition protocol at baseline and follow-up. The mean age of subjects (autism and control) was 10.9 years (range: 8.1–12.9 years) at baseline and 12.8 years (range: 11.0–15.4 years) at follow-up. The mean time difference between the two scans was 2.1 years with a range of .7 to 3.7 years. No difference in this interval period was observed between individuals with autism (2.3 years) and control subjects (2.0 years; $t = 1.414$; $df$ 32; $p = .167$)

All scans were obtained on the same GE 1.5-Tesla Signa whole-body MRI system (GE Medical Systems, Milwaukee, Wisconsin). A T1-weighted SPGR sequence was first acquired with the following parameters: slice thickness = 1.5 mm, slice numbers = 124, echo time (TE) 5 msec, repetition time (TR) 25 msec, flip angle 40°, number of excitations (NEX) 1, field of view (FOV) 24 cm, matrix 256 × 192. Proton density (PD) and T2-weighted images were then obtained using a single double-echo protocol with the following parameters: slice thickness = 5 mm, TE 17 msec for PD or 102 msec for T2, TR 2500 msec, NEX 1, FOV 24 cm, matrix 256 × 192, total slices = 24. All images were obtained in the coronal plane. MRI data were identified by scan number to retain blindness and analyzed using Brain Research: Analysis of Images, Networks and Systems software (BRAINS) while applying previously published methodologies of total brain volume

(TBV) measurements (24). The image processing was performed on a SGI workstation (Silicon Graphics, Mountain View, California) using the BRAINS2 (University of Iowa, Iowa City, Iowa) software package.

The image data were normalized to standard Talairach stereotactic three-dimensional space (25) by identifying six brain-limiting points (anterior, posterior, superior, inferior, left, and right); the anterior-posterior commissure line specified the x axis, a vertical line rising from the x axis through the interhemispheric fissure specified the y axis, and a transverse orthogonal line with respect to x and y coordinates specified the z axis. Registration was performed by aligning the T2 and PD images with a resampled T1 image and then resampling the T2 and PD images themselves (24). After normalization to a standard three-dimensional space, the pixels representing gray matter, white matter, and cerebrospinal fluid were identified using a segmentation algorithm applied to the T1, T2, and PD image sequences (26).

Volumetric measurements were performed using the BRAINS2 masks as generated by a neural network and corrected by manual tracing (ICC $>$ .9). TBV was defined as the cerebrum, cerebellum, and brainstem and excluding cerebrospinal fluid (24). The initial step of surface analysis in BRAINS is the creation of a triangle-based isosurface using the parametric center of the cortex as the outer boundary of the brain (27). CT is calculated from vectors that are normal to each triangular surface, and the shortest distance to the 50% gray matter and 50% white matter is defined as the thickness. Taking into consideration that the triangle isosurface lies at the parametric center of gray matter (approximately half of the actual CT), the values are multiplied by two (27).

### Data Analysis

Between-group differences on demographic data and changes over time were analyzed with two-tailed Student's *t* tests and were reported as means and standard deviations (M ± SD). Within-group differences were analyzed using paired test. To examine group differences in neurobiologic measurements multivariate analysis of variance (MANOVAs) were computed with subject group (autism vs. control) as the independent variable and brain volume (total brain volume, total gray matter, total white matter) or CT thickness (frontal, temporal, parietal, occipital, and total) as dependent variables in separate analyses. Because FSIQ differed between the autism and control groups, the same analyses as described earlier were computed except that FSIQ was included as a covariate (MANCOVA) to determine whether effects could be accounted for by this group difference.

This multivariate approach was used rather than separate univariate tests because it provides good control over Type I error rates. However, because the study is a novel examination of longitudinal changes in brain volume and CT and MANOVA approaches can increase Type II errors, univariate tests (analyses of variance and covariance with FSIQ as the covariate) were computed when marginally significant multivariate tests ($p < .10$) were present. This balances Type I and II errors and identifies effects that may be promising for follow-up in future research. To assist in interpretation of these univariate follow-up tests, effect sizes were computed (autism vs. control) using Cohen's *d* (28).

Pearson's correlation coefficients were used to examine the association between neuroimaging measurements and FSIQ. Spearman's rho correlation coefficients were used to examine the relationship between changes in CT measurements over time and ADI-R. Partial correlations were also applied to examine associations between neurobiologic and clinical measures while controlling for confounding factors. Probability figures were considered significant if they achieved significance at conventional levels (i.e., $p < .05$). The false discovery rate approach was used to correct for multiple comparisons whenever multiple univariate follow-up tests or correlations were considered (29,30).

## Results

No differences were observed between participants with autism and the control group on any of the demographic characteristics except for FSIQ (Table 1). Previously published baseline data from a subsample of this group revealed an increase in CT in children with autism when compared with control subjects (17). Changes over time were examined and differences in gray matter volumes were observed (Δ = Time 02 − Time 01) with significantly more decrease in gray matter volume in the autism group compared with control subjects. Similar changes were found in CT in the whole brain and in several brain regions including the temporal lobe (Table 2). When accounting for multiple comparisons, differences between the two groups were lost except for changes in occipital CT. Differences between the two groups were no longer significant after controlling for FSIQ (Table 2). Within-group differences revealed several differences in structural measurements in both groups (Table 3). The MANOVA revealed no significant patient,

**Table 2.** Changes in Cortical Thickness Between Time 01 and Time 02 (Δ = Time 02 − Time 01 in the Autism Group and Control Subjects

| | Autism ($n$ = 18) | | Controls ($n$ = 16) | | ANOVA $df$ (1,33) | | | ANCOVA $df$ (2,33) | |
|---|---|---|---|---|---|---|---|---|---|
| Brain Structure Measurements | Mean Δ | SD | Mean Δ | SD | $F$ | $p$ | $D$ | $F$ | $p$ |
| Brain Volume (cc) | | | | | | | | | |
| TGM | −11.0 | 23.8 | 4.2 | 18.3 | 4.295 | .046 | .73 | 3.828 | .059 |
| TWM | 12.5 | 33.1 | 10.3 | 18.1 | .058 | .811 | .09 | 1.114 | .299 |
| TBV | 1.5 | 33.8 | 14.5 | 19.9 | 1.797 | .190 | .19 | .263 | .612 |
| Cortical Thickness (mm) | | | | | | | | | |
| Frontal Lobe | −.5 | .5 | −.2 | .4 | 3.558 | .068 | .67 | 1.602 | .215 |
| Temporal Lobe | −.3 | .6 | .1 | .4 | 4.785 | .032 | 1.05 | 2.909 | .098 |
| Parietal Lobe | −.6 | .7 | −.3 | .5 | 2.471 | .126 | .56 | .253 | .619 |
| Occipital Lobe | −.5 | .4 | −.1 | .3 | 8.680 | .006 | .80 | 1.554 | .222 |
| Total Brain | −.5 | .5 | −.1 | .4 | 4.865 | .035 | .80 | 1.867 | .182 |

ANCOVA, analyses of covariance while controlling for full-scale IQ; ANOVA, analyses of variance; d, effect size; TGM, total gray matter; TBV, total brain volume; TWM, total white matter.

**Table 3.** Volumes and Cortical Thickness at Time 01 and Time 02 in the Autism Group and Control Subjects

| | Autism 1 | | Autism 2 | | Paired *t* Test *df* (17) | | Control 1 | | Control 2 | | Paired *t* Test *df* (15) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Structures | M | SD | M | SD | *t* | *p* | M | SD | M | SD | *t* | *p* |
| Volume (cc) | | | | | | | | | | | | |
| TGM | 867.48 | 92.91 | 856.47 | 81.08 | 1.96 | .066 | 835.04 | 65.15 | 839.24 | 64.17 | −.92 | .371 |
| TWM | 478.48 | 43.33 | 491.03 | 51.09 | −1.6 | .13 | 483.24 | 36.63 | 493.54 | 29.33 | −2.28 | .038 |
| TBV | 1345.96 | 129.42 | 1347.49 | 126.21 | −.2 | .85 | 1318.27 | 93.41 | 1332.79 | 88.10 | −2.91 | .011 |
| Cortical Thickness (mm) | | | | | | | | | | | | |
| Frontal | 5.34 | .27 | 4.82 | .21 | 4.04 | .001 | 5.14 | .19 | 4.94 | .15 | 2.011 | .063 |
| Temporal | 5.06 | .32 | 4.72 | .26 | 2.23 | .039 | 4.56 | .15 | 4.62 | .15 | −.68 | .509 |
| Parietal | 5.02 | .33 | 4.42 | .19 | 3.92 | .001 | 4.66 | .15 | 4.38 | .19 | 2.53 | .023 |
| Occipital | 4.24 | .28 | 3.78 | .18 | 5.27 | .000 | 3.98 | .17 | 3.84 | .08 | 1.90 | .077 |
| Total Brain | 4.98 | .26 | 4.52 | .19 | 3.99 | .001 | 4.70 | .13 | 4.54 | .14 | 1.76 | .099 |

TGM, total gray matter; TWM, total white matter; TBV, total brain volume.

time, or patient by time interaction for volumetric measurements ($p = .124$) or CT ($p = .099$). No changes in the level of significance were observed when multivariate analyses were conducted while controlling for FSIQ (volumetric measurements: $p = .324$; CT: $p = .161$). No correlations were found between changes in CT over time and FSIQ in either group except in the occipital lobe in the control group ($r = .535$, $p = .033$). No associations were found between changes in TBV and FSIQ in either group.

The examination of the relationships between changes in CT over time (Δ = Time 02 − *Time* 01) and clinical features revealed several associations. However, when applying the false discovery rate approach to control for multiple comparisons, only two correlations remained significant: the relationship between change in frontal lobe CT and lack of socioemotional reciprocity ($R = -.506$; $p = .035$) (Figure 1) and the association between change in temporal lobe CT and repetitive behaviors/stereotyped patterns ($R = -.484$; $p = .044$). When controlling for FSIQ, these relationships became less significant with the former showing a trend (partial $R = -.445$; $p = .073$).

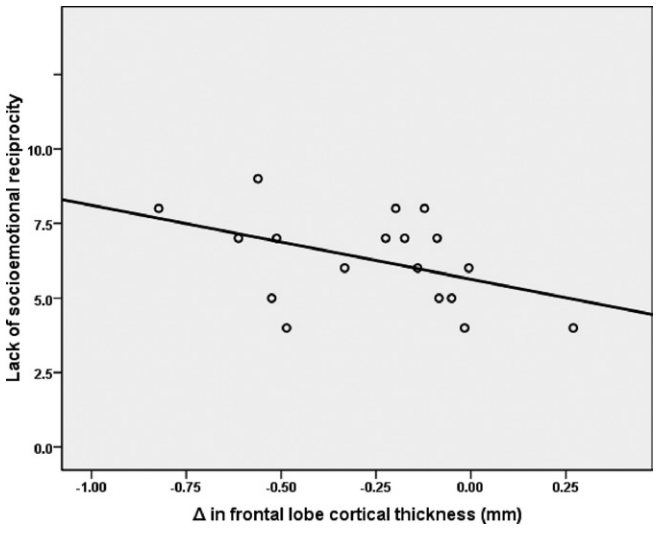


**Figure 1.** Relationship between lack of socioemotional reciprocity as measured by the Autism Diagnostic Interview—Revised and changes in cortical thickness over time (Δ Time 02 − Time 01) in the frontal lobe in the autism group ($R = -.506$; $n = 18$; $p = .035$).

## Discussion

In this longitudinal investigation, we measured developmental changes in brain volume and in CT in a group of children aged 8–12 years with autism compared with sex and age group-matched healthy control subjects. A decrease in gray matter volume was observed over time and appeared to be related, at least partially, to a decrease in CT. Associations between developmental changes in CT and several clinical features were also observed. However, most of the significant findings disappeared when accounting for multiple comparisons which could be related to the small sample size. Only differences in occipital lobe CT survived a multiple comparison correction, suggesting that occipital lobe changes are more pronounced and diffuse, whereas changes in the other lobes were subtle and localized. Analyses were also conducted while accounting for FSIQ, which made results less significant. This strategy is controversial because limited cognitive abilities are considered an integral part of the disorder and controlling for IQ differences is like accounting for the severity of symptoms. However, controlling for cognitive level might be necessary in light of the recent evidence suggesting the existence of associations between overall and subcomponents of human intelligence and CT in specific brain regions such as prefrontal and temporal lobe cortices (31,32). Despite these observations, these preliminary findings emphasize that the time course of brain development rather then the final product is most disturbed in autism (33).

Over the past two decades, several structural neuroimaging investigations have reported an increase in brain size in autism (2,34,35). More recently, additional evidence has emerged pointing to the presence of brain enlargement in individuals with autism and, more important, to the existence of age-related volumetric changes involving total brain as well as specific cerebral lobes (1–5). Our findings in this investigation, although preliminary, are consistent with some but not all of these observations. No differences in brain volume were observed here, which is inconsistent with most previous studies of brain volume. This discrepancy could be related to the small sample size or the age of participants. However, our result of age-related changes in gray matter volume is concordant with several cross-sectional studies providing indirect evidence of abnormal developmental trajectories in autism (1–5).

In this investigation, preliminary evidence of a decrease in CT over time was observed in the autism and control groups. This decrease involved several brain regions including the frontal,

parietal, and occipital lobes. Our observations are consistent with previous investigations in typically developing children, between the ages of 5 and 11 years, reporting cortical thinning over large brain areas such as the right dorsolateral frontal and bilateral occipitoparietal cortices (36). Interestingly, an increase in CT over time was observed here in the temporal lobe in healthy control subjects but not in the autism group, which is concordant with findings of gray matter thickening in left and right posterior perisylvian regions of Wernicke's area in neuro-typical children (36). These structural alterations in the temporal lobe suggest the existence of an aberrant cortical development in autism and might explain the previously reported functional abnormalities in this structure (37).

Several clinical features including social deficits and repetitive behaviors were associated with changes in CT over time in different brain regions in the autism group. Neuroanatomic measurements in the frontal lobe were associated with social functioning. This observation is consistent with recent evidence implicating the mirror neuron system in autism (18,38) and with investigations reporting on abnormalities in the frontal lobe circuitry in this disorder (18,39). In this study, relationships were also observed between repetitive/stereotyped patterns of behaviors and changes in CT in the temporal lobe in the autism group. This association is concordant with a structural MRI study that found partial correlations between repetitive behavior measures and volumes of multiple regions including those in the temporal lobe (40). This relationship is also supported by recent evidence from neuroimaging studies examining individuals with obsessive-compulsive disorder, which is characterized by repetitive/ritualistic behaviors, and structural abnormalities in the temporal lobe (41,42). Finally, all the correlations described earlier appear to indicate that excessive thinning of the cortex was associated with severe symptomatology independent of the clinical feature and the brain region involved. Elevated scores on the ADI-R subdomains correlated with decreases in CT. These observations suggest that changes in CT over time might serve as an indicator of illness severity, but further studies are definitely warranted.

Our preliminary observations point to an excessive decrease in gray matter volume and CT in individuals with autism during a period of development when competitive elimination of synapses and dendritic/axonal arborization represent the main neuronal structural activity. These findings should be examined in light of the recent evidence suggesting that changes in gray matter volumes, may, at least in part, reflect intracortical myelination and the resulting partial volume effect (43). Changes in CT over time observed in this investigation could reflect alterations in the gray-white matter boundary related to myelination and changes in the cortical mantle itself. Hence, increase in white matter volume could lead to cortical thinning because of increased myelination between gray and white matter in the periphery of the cerebrum. However, in this investigation no differences in white matter volumes in the two groups were observed at baseline, at follow-up, and over time. This observation suggests that the excessive decrease in CT observed in the autism group is related to decrease in gray matter possibly related to alterations of the cortical gray matter during the peri-adolescence period. An excessive decrease in any of the various elements of the cortical mantle (cell bodies, neuropil, and synapses of neurons) in isolation or in combination could lead to the findings observed here. Consequently, neuropathologic studies of individuals with a wide age range are therefore warranted to assess the basic histological features of the cortex in autism in different age groups to determine the exact ultrastructural underpinnings of these alterations. In the absence of white matter volume changes, these alterations suggest the existence of alterations intrinsic to gray matter in autism during the peripubertal period and could be related to synaptic development and maturation. This observation is supported by evidence from genetic studies reporting a link between autism and several genes implicated in synapse formation and dendritic spine maturation, such as SHANK3 (44).

The neurobiologic underpinnings of cortical changes observed in this investigation remain to be elucidated. Previous research has implicated several neurotrophins, including brain-derived neurotrophic factor (BDNF), in neuronal development (45). Several studies have reported abnormal serum levels of BDNF in individuals with autism throughout the life span (46–49). Interestingly, age-related differences have been reported with a recent study describing lower levels of BDNF in the serum of children with autism aged less than 9 years compared with adolescents, adults, and control subjects (49). These developmental changes might be related to the altered peripubertal cortical organization observed in our study. Additionally, the serotoninergic system has also been implicated in autism (50). This system might be playing a role in the abnormal brain development in autism either directly or indirectly by regulating neuronal development signaling factors for neurotrophins (51). However, although mounting evidence is emerging linking abnormalities in neurotrophins and serotonin to autism, it is not clear whether these alterations are the primary cause or secondary to the altered cortical changes or just an associated process. Finally, structural and neurobiologic abnormalities should also be examined in light of the fundamental role that genetic and environmental factors play in influencing brain structure, including CT, during development (52,53). This is particularly true for autism for which recent evidence has been reported linking gene polymorphisms to cortical enlargement (54) and gray matter overgrowth (55).

Findings from this preliminary study should be examined in light of several methodologic limitations. First, lower-functioning individuals and females were not included in this study, which might limit the generalizability of its findings. Future investigations of this nature will have to address possible sex-related differences in the development of cortical structures and associated severity of clinical features. Second, only two MRI scans were obtained from each participant, and additional scans obtained over time would have provided more data points to allow for more accurate conclusions. Additionally, scans were obtained on a 1.5-Tesla GE machine and acquisition of images on a higher field strength scanner might have yielded different findings. Third, the exclusion of participants younger than 8 years of age is limiting because most brain development occurs before this age. Therefore, final interpretations from this study should not be made before findings from studies of very young children are completed. Fourth, the morphometric program used generated global and lobar measures of volume and CT, which could explain the loss of significance when controlling for multiple comparisons and confounding factors. The lack of topographic specificity limits the interpretations of the findings and warrants future studies applying more advanced programs to allow the examination of structural alterations at the gyral or sulcal level or on a voxel-by-voxel basis. Fifth, information on the variability of outputs and segmentations of various tissue types from MRI images taken at the two time points (baseline and follow-up) should have been measured to provide assurance that data from the two sessions are equivalent. Finally, additional adjustment of

the *p* value might be necessary in light of the number of comparisons conducted.

In summary, this longitudinal MRI study is, to our knowledge, the first investigation to examine longitudinal changes in brain volume and CT in children with autism. Developmental structural changes were observed and relationships were found with clinical measurements. This investigation provides preliminary evidence of the age-related changes in gray matter volume and CT during the crucial peripubertal period of development in males with autism. However, the neurobiologic underpinnings of these changes and the genetic control of these mechanisms remain to be elucidated. Future longitudinal studies applying multimodal imaging techniques while examining genetic influences by assessing gene polymorphisms are necessary to evaluate developmental trajectories across the life span in this disorder.

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