

# Gray and White Matter Brain Chemistry in Young Children With Autism

Seth D. Friedman, PhD; Dennis W. W. Shaw, MD; Alan A. Artru, MD; Geraldine Dawson, PhD; Helen Petropoulos, BE; Stephen R. Dager, MD

**Context:** The brain pathophysiological abnormalities underlying autism remain unclear. Neuroimaging and histological studies suggest cellular abnormalities early in the course of the disease.

**Objective:** To measure the in vivo chemical profile of gray and white matter tissues in autism.

**Design:** Cross-sectional spectroscopic imaging study comparing 3- to 4-year-old children with autism spectrum disorder (ASD) with age-matched comparison groups of children with delayed development (DD) and typical development (TD).

**Setting:** The University of Washington Diagnostic Imaging Sciences Center, Seattle.

**Participants:** Forty-five 3- to 4-year-old children with ASD, 12 age-matched children with DD, and 10 age-matched children with TD.

**Main Outcome Measures:** Estimates of gray and white matter concentrations for choline-containing compounds (Cho), creatine plus phosphocreatine, N-acetylaspartate (NAA), and myo-inositol (mI). Transverse relaxation times for Cho, creatine plus phosphocreatine, and NAA expressed relative to control subjects with TD were examined to evaluate tissue compactness.

**Results:** The children with ASD demonstrated decreased gray matter concentrations of Cho ( $P < .001$ ), creatine plus phosphocreatine ( $P = .02$ ), NAA ( $P = .02$ ), and mI ( $P = .008$ ) compared with children with TD. Gray matter Cho transverse relaxation was also prolonged for the ASD sample compared with the TD group ( $P = .01$ ). The children with ASD demonstrated significantly decreased levels of Cho ( $P = .04$ ) and mI ( $P = .008$ ) and trend-level NAA ( $P = .09$ ) in gray matter compared with the DD group. For white matter, both children with ASD and children with DD showed a similar pattern of NAA and mI level decreases (for children with ASD vs children with TD: NAA,  $P = .03$ ; mI,  $P = .04$ ; for children with DD vs children with TD, NAA,  $P = .03$ ; mI,  $P = .07$ ). In several analyses, cerebral volume contributed significantly as a covariate.

**Conclusions:** Reduced gray matter chemical concentrations and altered Cho transverse relaxation, in a pattern distinct from that in children with DD, suggest decreased cellularity, or density, at this early time point in ASD. Possibly reflecting shared developmental features, white matter results were common to ASD and DD groups. The relationship between cerebral volume and neurochemistry at this early time point may indicate processes related to unit scaling.

*Arch Gen Psychiatry. 2006;63:786-794*

## Author Affiliations:

Departments of Radiology (Drs Friedman, Shaw, and Dager and Ms Petropoulos), Anesthesiology (Dr Artru), Psychology (Dr Dawson), Psychiatry (Dr Dager), and Bioengineering (Dr Dager), University of Washington School of Medicine, Seattle.

**A**UTISM SPECTRUM DISORDER (ASD) is characterized by abnormal social interactions, impaired communication, and behavioral stereotomies.<sup>1</sup> Although the etiology of autism remains unclear, emerging evidence from neuroimaging and histopathological post-mortem studies suggests that early alterations that change over time may be responsible.

From magnetic resonance imaging (MRI) and head circumference studies, there is evidence to suggest that cerebral volume is not enlarged in ASD at birth (for review, see the article by Redcay and Courchesne<sup>2</sup>). Increased brain size is observed

between approximately 2 and 4 years of age and may reflect accelerated brain growth patterns (for review, see the article by Redcay and Courchesne<sup>2</sup>). Across the development in ASD, cross-sectional studies have suggested that this early overgrowth halts in later childhood, affecting gray and white matter, brain lobes, and nuclei differently (for review, see the article by Courchesne et al<sup>3</sup>). The way in which gray and white matter or regional volumes scale to the overall cerebral volume may also be important, with several studies<sup>4,5</sup> demonstrating that correcting for cerebral volume modifies group differences. Taken together, these morphological results build on the first observation of

macrocephaly in autism<sup>6</sup> and associate these macroscopic findings to mechanisms expressed in the first few years of the disease.

The 2 most replicated postmortem findings in ASD are small, densely packed cells in the medial temporal lobe (hippocampal regions CA1 and CA4) and a reduction in Purkinje cells in the cerebellum (for review, see the articles by Palmen et al<sup>7</sup> and Bauman and Kemper<sup>8</sup>). Certain alterations such as enlargement of cells in the nucleus of the diagonal band of Broca are found in children (aged <13 years), but in adults, the cells are reduced in both size and number, suggesting developmental changes.<sup>8</sup> Other cellular abnormalities appear to have variable localization and expression,<sup>9</sup> consistent with the heterogeneous clinical expression of ASD.<sup>10</sup> Cortical abnormalities have been described in a number of ASD samples.<sup>7-9,11,12</sup> Described findings include thicker, abnormal cortical laminar patterns, increased neuron density, abnormal orientation of pyramidal cells,<sup>11</sup> minicolumnar abnormalities,<sup>12</sup> and neuroinflammation.<sup>9</sup> Although not all of these processes, eg, inflammation,<sup>9</sup> are solely confined to gray matter, further work is required to evaluate in vivo distributions of such abnormalities early in the disease course.

In vivo proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS) provides a noninvasive method for probing histochemical features in vivo. It has been used to detect abnormalities in brain regions that appear normal by MRI as well as to elucidate the pathological findings underlying MRI-visible abnormalities (for review, see the article by Ross and Michaelis<sup>13</sup>). In brain tissue, the concentration and mobility of several low-molecular-weight chemicals such as choline-containing compounds (Cho), creatine plus phosphocreatine (Cre), *N*-acetylaspartate (NAA), *myo*-inositol (mI), glutamate, glutamine,  $\gamma$ -aminobutyric acid, and lactate can be sampled using <sup>1</sup>H-MRS. As a brief overview, chemicals are reviewed in turn. The Cho in the <sup>1</sup>H-MRS spectrum are observed as a single peak, or a singlet. However, as is evident from phosphorous studies with proton decoupling, at least 4 major chemicals related primarily to membranes and myelin contribute to the bulk of the <sup>1</sup>H-MRS Cho signal. Phosphorylethanolamine and phosphorylcholine are the primary components, with lesser contributions from glycerophosphorylethanolamine and glycerophosphorylcholine.<sup>14</sup> The peak identified as Cre in the <sup>1</sup>H-MRS spectrum is composed of both creatine and phosphocreatine, which remain in equilibrium and serve as the storage and use mechanism for high-energy phosphate.<sup>13</sup> *N*-acetylaspartate, considered a putative neuronal marker, contains a small contribution from *N*-acetylaspartylglutamate at 1.5 T.<sup>15</sup> *N*-acetylaspartate functions as a critical osmolyte involved in neuronal-glial homeostasis.<sup>16</sup> *Myo*-inositol, another resonance that shares some functions with Cho, is an important regulator of brain osmotic balance and a precursor for phosphoinositides involved in the cellular membrane-based second messenger system.<sup>17-19</sup> Glutamine, produced by astrocytes and in turn shuttled to neurons for producing glutamate, the major excitatory neurotransmitter in brain, is challenging to resolve without specialized techniques.<sup>20</sup> Glutamine and glutamate overlap

in frequency in the chemical spectrum at 1.5 T and have a complicated multiplex, or multiplet, shape.  $\gamma$ -Aminobutyric acid, the major inhibitory neurotransmitter, also overlaps in frequency with glutamate and glutamine, although lesser in concentration. Because of the difficulty in separating these neurochemicals and the low abundance of  $\gamma$ -aminobutyric acid, many investigations report a combination of these chemicals, commonly as glutamate plus glutamine. Lactate, a major energy substrate for neurons that is produced primarily in glial cells, can also be measured in brain, although levels at rest in healthy brain are quite low.<sup>21</sup>

The MRS chemical measurements are expressed as chemical ratios (eg, NAA-Cre ratio) or quantified using an internal (eg, brain water) or external (eg, solution of known concentration) reference signal. Altering acquisition delay (echo time [TE]) results in distinct changes in the chemical spectrum that impact neurochemical measurement. Shorter TEs (<30-ms) reduce some potential confounds associated with chemical quantification, although certain chemicals, such as lactate, can be obscured by lipid and macromolecule signals. At longer TEs (136-ms or 272-ms), these macromolecule and lipid peaks are less visible, although other chemicals, such as mI, are also edited out of the spectrum. Without broad baseline peaks, longer TEs make line fitting more straightforward. However, because signals have had more time to evolve (or decay), group differences in relaxation, should they be present, can significantly bias neurochemical measurement. The decay in chemical intensity with time can be used to obtain a measure of transverse relaxation (T<sub>2</sub>). Although many factors impact T<sub>2</sub>,<sup>22</sup> we used this measurement in our prior work<sup>23</sup> as a probe of tissue compactness.

Most MRS studies of autism have used single-voxel techniques that acquire chemical information from a single region of tissue, typically of large volume to obtain sufficient signal-noise ratios. The first published MRS study<sup>24</sup> of autism sampled a right parieto-occipital white matter region at a long TE and found no alterations in the ratios (eg, NAA-Cre, Cho-Cre, and NAA-Cho ratios) in a sample of children and adolescents with autism. Using a short-TE acquisition method, a subsequent single-voxel MRS study detected a decreased level of NAA (referenced to brain water) in the right medial temporal lobe region and left cerebellar hemisphere in a childhood to early adult sample<sup>25</sup> and an expanded sample.<sup>26</sup> From a large sample of children ranging in age from 2 to 21 years who were studied at an intermediate TE (136 milliseconds), reduced NAA levels were demonstrated in the temporal lobes bilaterally, although not in other regions (frontal, parietal, temporal, brain stem, or cingulate).<sup>27</sup> In contrast, a single-voxel study of high-functioning adults with Asperger syndrome also conducted at an intermediate TE (136 milliseconds) found increased levels of all of the chemicals (Cho, Cre, NAA as a ratio to water) in a right frontal region but not within a medial parietal region.<sup>28</sup> Specifically sampling a white matter region (left centrum semiovale) at a short TE (30 milliseconds), no chemical concentration differences were found for a recent sample of children with autism.<sup>29</sup>

Magnetic resonance spectroscopic imaging techniques used to simultaneously acquire 2-dimensional or 3-dimensional voxel arrays for regional chemical assess-

ment have been used in 2 ASD studies to date. Using short-echo (20-ms) and long-echo (272-ms) proton echo-planar spectroscopic imaging (PEPSI)<sup>30</sup> and water referencing, we examined a large sample of 3- to 4-year-old children with ASD compared with age-matched control groups with delayed development (DD) and typical development (TD). The ASD sample exhibited a regional pattern of decreased neurochemical concentrations that included widespread reductions in levels of Cho, Cre, NAA, and mI.<sup>23</sup> Prolonged chemical T2 expressed as a percentage relative to the TD group (T2r) was also observed in many regions.<sup>23</sup> A subsequent MRS imaging study<sup>31</sup> at a long TE (272 milliseconds) used a multisection approach and quantification based on acquisition parameters to examine 22 children and adolescents with autism compared with age-matched control subjects. In that study, a decreased Cho level was found within the left anterior cingulate, left caudate, and occipital regions, and increased levels of Cho and Cre were found within the right caudate nucleus.

In our prior work, results of regional reductions in levels of NAA and other tissue-based chemicals were in contrast to our initial hypothesis. Our initial hypothesis had been that, in conjunction with findings of cerebral enlargement, the children with ASD would exhibit elevated NAA levels and reduced T2r reflecting an overproliferation of cells or a reduction in normal pruning processes.<sup>23</sup> What could not be ascertained from that work was whether gray matter or white matter was specifically affected in this young ASD sample. In the current study, based on findings of generalized histopathological alterations in gray matter, we hypothesized that gray matter chemicals are selectively altered in ASD. To test this hypothesis, linear regression techniques<sup>32,33</sup> were applied to our previously published sample of 3- to 4-year-old children with ASD compared with the age-matched DD and TD control groups. To evaluate the association between brain size and these chemical measures, cerebral volume was used as a covariate. To evaluate ASD subgroups (autistic disorder [AD] and pervasive developmental delay not otherwise specified [PDD-NOS]) found in our prior work<sup>5</sup> to have different amygdala volumes, exploratory analyses were planned as indicated by significant ASD-TD main effects.

## METHODS

### PARTICIPANTS

Clinical characteristics of the children participating in this research have been described in detail elsewhere.<sup>5,23</sup> For these analyses, 45 children with ASD (38 boys, 7 girls; mean±SD age, 47.4±4.2 months) were compared with 12 children with DD (5 boys, 7 girls; mean±SD age, 47.5±6.1 months) and 10 children with TD (8 boys, 2 girls; mean±SD age, 46.6±4.5 months). For exploratory analyses, the ASD sample was further differentiated into AD (n=29; 26 boys, 3 girls; mean±SD age, 46.9±4.3 months; age range, 38-54 months) and PDD-NOS (n=16; 12 boys, 4 girls; mean±SD age, 48.2±4.0 months; age range, 42-54 months) subgroups based on clinical characteristics as previously described.<sup>5</sup>

Children were recruited from local parent advocacy groups, preschools, the Department of Developmental Disabilities, Seattle, Wash, clinics and hospitals in the greater Seattle area, and

the University of Washington, Seattle, infant and child subject pool. Written parental or guardian informed consent, approved by the University of Washington internal review board, was obtained for each child participating in the study. Children in the ASD group received a diagnostic evaluation that included administration of the Autism Diagnostic Interview-Revised,<sup>34</sup> a structured clinical interview with the parent, and the Autism Diagnostic Observation Schedule-Generic,<sup>35</sup> a structured clinical observation scale that is scored for the severity of autism symptoms. Both instruments assess symptoms of autism listed in the DSM-IV.<sup>36</sup> Children with DD and with TD were also administered the Autism Diagnostic Observation Schedule-Generic. These children did not meet criteria for either AD or ASD on the Autism Diagnostic Observation Schedule-Generic or based on a clinical judgment using DSM-IV criteria, and they did not show elevated symptoms of autism on these measures.

Children with ASD and DD were assessed using the Mullen Scales of Early Learning<sup>37</sup> and the Vineland Adaptive Behavior Scale.<sup>38</sup> Mullen Scales of Early Learning group mean±SD scores on the primary measure, composite standard score, were as follows: for children with ASD, 59.2±15.9; and for children with DD, 54.9±6.3 (1 child with DD did not receive a Mullen Scales of Early Learning assessment).

### MRI AND SPECTROSCOPY ACQUISITION

Children with ASD and DD were imaged during continuous intravenous infusion of propofol at 180 to 220 µg/kg per minute. Studies of children with TD were performed late at night during sleep. Eight children with TD were presedated with 25 mg of Benadryl (Pfizer, Inc, New York, NY) administered orally and by the parent on an optional basis if the child previously had experienced sedation when given this agent.

The PEPSI studies were performed using a clinical 1.5-T GE Signa whole-body scanner (General Electric Medical Systems, Milwaukee, Wis) as previously described.<sup>23</sup> A 3-dimensional spoiled gradient recalled echo imaging sequence (repeat time, 33.3 milliseconds; TE, 30 milliseconds; flip angle, 30°; field of view, 22 cm; matrix, 256×256; section thickness, 1.5 mm [3 mm zero filled to 1.5 mm during acquisition]) was acquired in the coronal plane and used for tissue segmentation.

Two contiguous PEPSI volumes were serially acquired, one centered at the top of the temporal lobes (the first axial section centered on the anterior commissure) and the other through the basal ganglia as previously described (TE, 20 milliseconds and 272 milliseconds; repeat time, 2000 milliseconds; spatial matrix, 32×32; nominal voxel size, 1 cm<sup>3</sup>; field of view, 22 cm; section thickness, 20 mm). Long-echo (272-millisecond) data analysis required a magnitude calculation. Both chemical TEs were referenced to a short-echo unsuppressed water scan for concentration calculation.<sup>23</sup> Lactate was measured with the 272-millisecond data to avoid inclusion of macromolecule resonances.

In 2 children with ASD and 3 with TD, only 1 slab was available for regression analyses because of poor data quality or because a child with TD awoke prior to completing the acquisition. For 1 child with TD, only a long-echo data set was acquired and analyzed for lactate.

Because of the smaller proportion of subjects (7 of 10 subjects) in the TD group having both slabs available, fewer voxels were available for the regression (eg, for NAA [TE, 20 milliseconds], the mean±SD number of voxels was 501±60 voxels for the ASD group, 460±50 voxels for the DD group, and 361±118 voxels for the TD group). To maximize data fidelity from each subject, all of the available voxels from each individual subject were used to generate estimates of compartmental concentrations and relaxation times.

## DATA PROCESSING

The high-resolution spoiled gradient recalled echo images were corrected for field inhomogeneity using the nonparametric nonuniform intensity normalization technique<sup>39</sup> and segmented using a Bayesian classifier.<sup>40</sup> Each tissue map was then spatially filtered to match the PEPSI point-spread function and reduced to match the PEPSI resolution ( $32 \times 32$ ). On a voxel-by-voxel basis, cerebrospinal fluid data were used for partial volume correction of chemical quantification and gray and white matter maps were used for regression calculations.

The PEPSI chemical images were analyzed as previously described using software developed at the University of Washington that uses the LCMoDel software package for spectral fitting<sup>41</sup> (for example spectra in a subject with ASD, see **Figure 1**). The T2r values derived from the paired short- and long-echo PEPSI data were determined for NAA, Cre, and Cho on a voxel-by-voxel basis. Using T2r is synonymous to using institutional units for chemical measurement, and although its use does not allow determination of absolute T2 differences between groups, the direction and magnitude of findings are comparable to absolute T2 measurements.

For each subject, regression analyses used all of the valid spectroscopy voxels to compare the fractional tissue volume (percentage of gray or white matter per voxel) with concentration and relaxation values using Matlab version 6.0 software for UNIX (Mathworks, Inc, Natick, Mass). This approach, similar to that used by other investigators,<sup>32,33</sup> takes advantage of the large number of voxel samples obtained using spectroscopic imaging to calculate estimates of gray or white matter neurochemistry (regression intercepts) (for an example data set from a single subject, see **Figure 2**).

## STATISTICAL ANALYSIS

No differences in the gray matter–white matter ratio within the spectroscopy slabs were found between groups (mean  $\pm$  SD gray matter–white matter ratio,  $0.67 \pm 0.03$  for the ASD group,  $0.68 \pm 0.04$  for the DD group, and  $0.65 \pm 0.07$  for the TD group;  $F_{2,67} = 1.79$ ;  $P = .18$ ). No group differences in age were found ( $F_{2,67} = 0.54$ ;  $P = .59$ ). Differences in sex distribution were present between groups (Fisher exact test,  $8.57$ ,  $df = 2$ ;  $P = .01$ ), reflecting a greater proportion of girls to boys in the DD sample. Because of this group difference, sex was included as a covariate in analyses of covariance performed in SPSS version 11.0 software for Macintosh (SPSS, Inc, Chicago, Ill). Cerebral volume was also included as a covariate because exploratory correlations revealed association to chemical measures (data not shown). Statistical significance for all of the tests was set at 2-tailed  $\alpha = .05$ . For main effects reaching significance, Tukey post hoc testing was used.

Follow-up analyses paralleling significant group main effects (ASD vs TD differences) were planned, stratifying the ASD group into AD and PDD-NOS subgroups.

## RESULTS

Descriptive statistics for the subject samples, concentration, and relaxation values (estimated marginal means  $\pm$  SEs) and statistical results are shown in **Tables 1**, **2**, and **3**. Cerebral volume and sex, entered as covariates, were significant where indicated. For significant main effects, post hoc results are described here.

## ASD GROUP VS TD GROUP

The ASD sample demonstrated decreased gray matter concentrations of Cho (17.4%), Cre (6.5%), NAA (4.8%), and mI (10.3%) compared with the TD group. The Cho T2r was elevated (12.4%) in gray matter in the ASD group compared with the TD sample. In white matter, the ASD group showed decreases in concentrations of NAA (5.1%) and mI (10.7%) compared with TD controls. After covarying for cerebral volume, no differences in white matter T2r were demonstrated between the ASD and TD groups (Table 3).

## ASD GROUP VS DD GROUP

In gray matter, the ASD sample demonstrated decreased Cho (6.7%) and mI (10.6%) concentrations compared with the DD group. Decreases in NAA concentrations were at the trend level (3.5%). After covarying for cerebral volume, white matter T2r main effects were not significant. For this reason, post hoc tests are not described, although the *P* values are included in Table 3 for descriptive purposes.

## DD GROUP VS TD GROUP

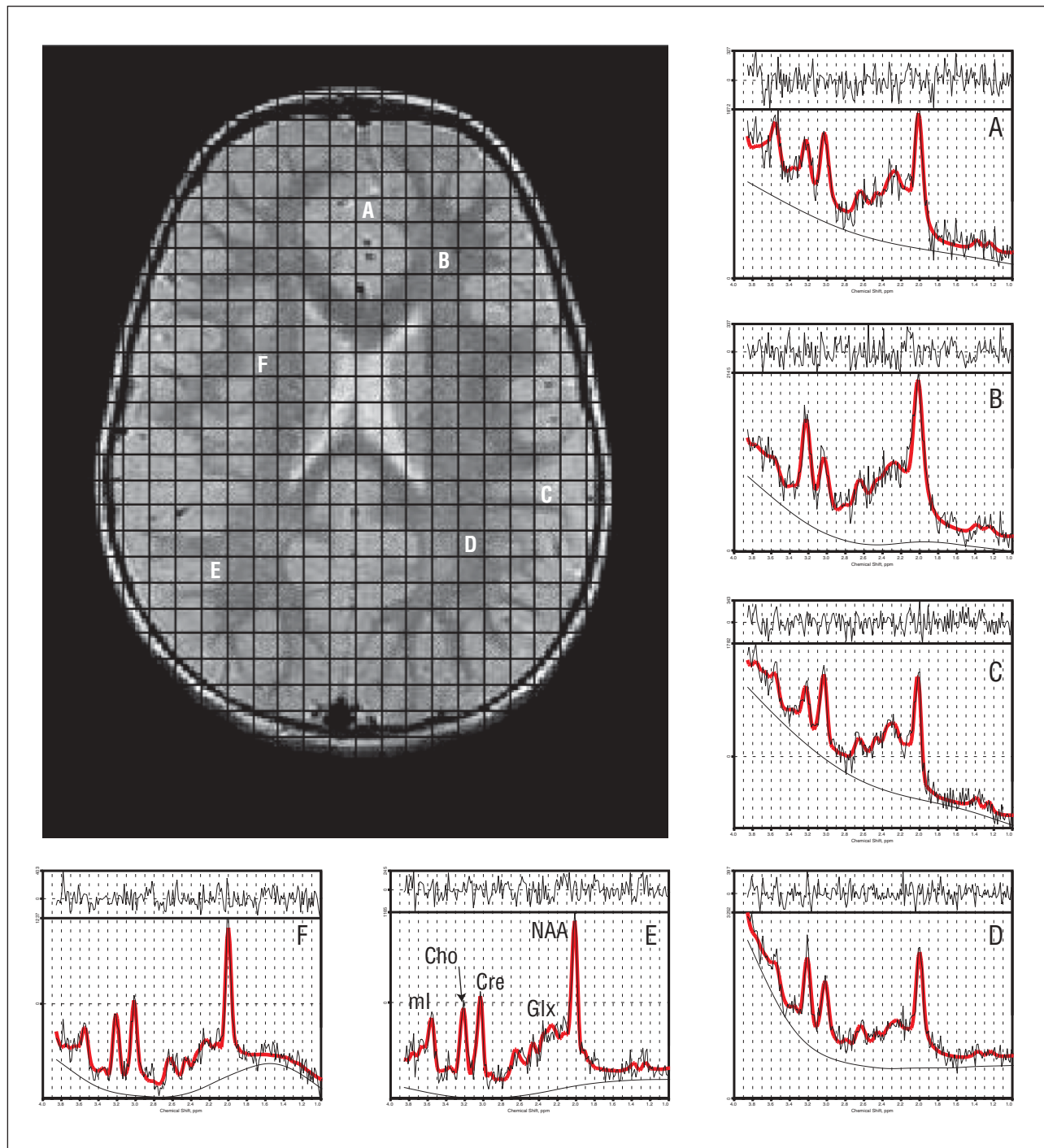
The DD sample demonstrated decreased gray matter concentrations of Cho (11.5%) compared with the TD group. In white matter, significant NAA (6.4%) and trend-level mI (12.3%) concentration decreases were observed for the DD group. After covarying for cerebral volume, white matter T2r main effects were not significant. Post hoc tests are not described, although the *P* values are included in Table 3 for descriptive purposes.

## AD SUBGROUP VS PDD-NOS SUBGROUP

For significant gray and white matter main effects, exploratory analyses were performed for the AD and PDD-NOS subgroups. Of all of the tests, only gray matter NAA concentration reached trend significance ( $F_{1,43} = 2.79$ ;  $P = .10$ ), with the PDD-NOS group demonstrating slightly higher (2%) NAA concentration than the AD sample (mean  $\pm$  SE NAA concentration,  $10.71 \pm 0.09$  for the PDD-NOS subgroup and  $10.52 \pm 0.07$  for the AD subgroup) (other statistics not shown). Since Mullen Scales of Early Learning scores between ASD groups revealed trend differences ( $t_{44} = 3.09$ ;  $P = .09$ ) (mean  $\pm$  SD Mullen Scales of Early Learning scores,  $56.2 \pm 13.4$  for the AD subgroup and  $64.6 \pm 18.0$  for the PDD-NOS subgroup), a further follow-up analysis included Mullen Scales of Early Learning performance as an additional covariate. Although Mullen Scales of Early Learning performance did not contribute significantly to the model ( $F_{1,41} = 0.04$ ;  $P = .84$ ), the model was no longer significant with this addition ( $F_{1,41} = 1.71$ ;  $P = .20$ ).

## COMMENT

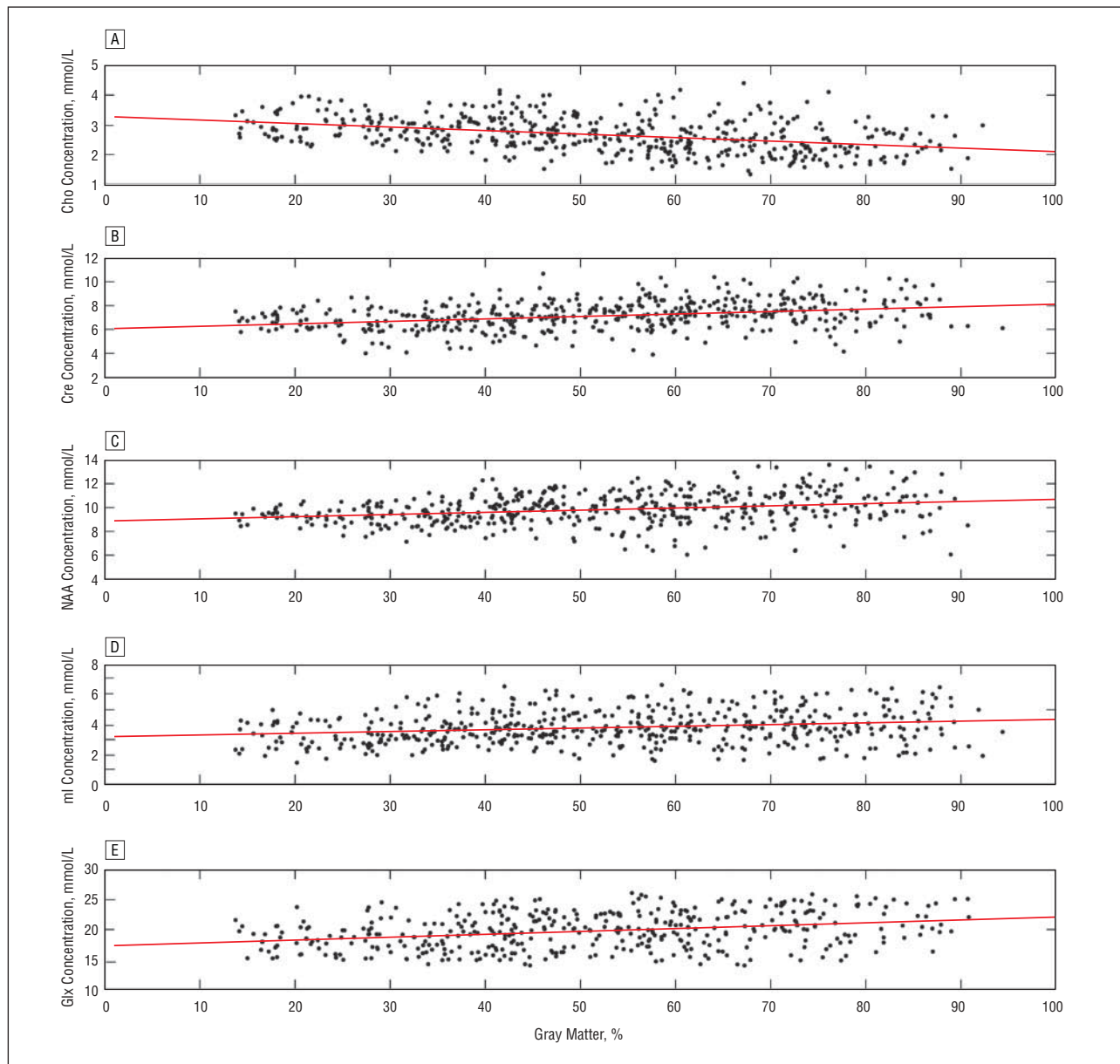
In this study, regression analytic techniques used to investigate gray and white matter chemical characteristics



**Figure 1.** A high-resolution prescription image with the proton echo-planar spectroscopic imaging grid overlaid is shown. Spectra from differing anatomical locations are shown, with corresponding spectra identified by letter. The  $32 \times 32$ -voxel array is overlaid on the central high-resolution image from a proton echo-planar spectroscopic imaging slab. Representative 20-millisecond spectra from gray and white matter voxels in a subject with autism spectrum disorder. ml indicates *myo*-inositol; Cho, choline-containing compounds; Cre, creatine plus phosphocreatine; Glx, glutamate plus glutamine; and NAA, *N*-acetylaspartate. For each spectrum, the lower plot shows the fitted baseline (smooth gray line), the raw data (thin gray trace), and the LCModel software fit (red line). At the top of each plot, the remaining signal following fitting, or residual signal, is displayed.

revealed a distinct pattern of gray matter chemical abnormalities in 3- to 4-year-old children with ASD compared with age-matched groups of children with DD and TD. The ASD sample demonstrated decreased gray matter Cho, Cre, NAA, and ml concentrations and prolonged gray matter Cho T2r compared with the TD sample. Compared with the DD group, the ASD sample

demonstrated decreased gray matter Cho and ml concentrations and a trend for decreased NAA concentration. Compared with the TD group, children with DD demonstrated reduced gray matter Cho concentration. Follow-up analyses comparing the ASD subgroups revealed a small but significant gray matter NAA concentration reduction in the AD subgroup compared with the



**Figure 2.** Example regression data set from the subject with autism spectrum disorder (the same subject referred to in Figure 1) showing chemical concentrations (in 20-millisecond spectra) by voxel gray matter–white matter fraction for choline-containing compounds (Cho) (A), creatine plus phosphocreatine (Cre) (B), *N*-acetylaspartate (NAA) (C), *myo*-inositol (ml) (D), and glutamate plus glutamine (Glx) (E). Regression lines computed from each chemical map are also plotted, demonstrating the derived estimates of white and gray matter chemical concentration (intercepts).

PDD-NOS subgroup. This finding may reflect the greater disease burden in the AD subgroup; further study is necessary to fully characterize the behavioral correlate of this group difference.

As shared features perhaps common to behavioral delay in general, both children with ASD and children with DD exhibited a similar pattern of white matter NAA and ml concentration decreases compared with the TD sample, although ml concentration differences were at the trend level for the DD group. In both gray and white matter, glutamate and glutamine or lactate concentration increases, not observed for the ASD or DD samples, militate against postulated mitochondrial dysfunction.<sup>21,42</sup> However, it should be noted that propofol used for both ASD and DD studies may reduce metabolic rate<sup>43</sup> and,

**Table 1. Descriptive Statistics for Subjects With Autism Spectrum Disorder, Delayed Development, and Typical Development**

Variable	Subjects With ASD	Subjects With DD	Subjects With TD
Subjects, No.	45	12	10
Age, mean ± SD, mo	47.4 ± 4.2	47.5 ± 6.1	46.6 ± 4.5
Age, range, mo	38-54	40-58	41-55
Male/female, No.	38/7	5/7	8/2
Anesthesia	Propofol	Propofol	Benadryl (n = 8)
Other medication	None	None	None

Abbreviations: ASD, autism spectrum disorder; DD, delayed development; TD, typical development.

**Table 2. Chemical Measures**

Tissue and Metabolite	ASD, Estimated Marginal Mean (SE)	DD, Estimated Marginal Mean (SE)	TD, Estimated Marginal Mean (SE)
Gray matter			
Cho	2.15 (0.03)	2.30 (0.07)	2.60 (0.07)
Cre	8.12 (0.10)	8.29 (0.21)	8.68 (0.21)
NAA	10.57 (0.09)	10.96 (0.20)	11.11 (0.20)
ml	4.42 (0.08)	4.95 (0.17)	4.93 (0.17)
Glx	21.07 (0.28)	20.41 (0.60)	21.48 (0.60)
Lac	0.78 (0.03)	0.78 (0.07)	0.76 (0.07)
White matter			
Cho	2.90 (0.04)	2.90 (0.10)	2.74 (0.10)
Cre	6.05 (0.08)	6.33 (0.17)	6.26 (0.17)
NAA	8.89 (0.09)	8.77 (0.19)	9.37 (0.19)
ml	3.42 (0.08)	3.36 (0.18)	3.83 (0.18)
Glx	14.91 (0.31)	15.18 (0.67)	16.38 (0.67)
Lac	0.56 (0.02)	0.51 (0.05)	0.61 (0.05)
T2r in gray matter			
Cho	12.37 (1.98)	5.76 (4.32)	0.00 (4.30)
Cre	-1.02 (1.10)	-3.57 (2.39)	0.00 (2.38)
NAA	-1.91 (0.85)	-2.91 (1.86)	0.00 (1.85)
T2r in white matter			
Cho	-6.32 (1.70)	-10.06 (3.67)	0.00 (3.69)
Cre	1.74 (1.29)	-4.44 (2.81)	0.00 (2.80)
NAA	2.62 (1.12)	-1.87 (2.43)	0.00 (2.42)

Abbreviations: ASD, autism spectrum disorder; Cho, choline-containing compounds; Cre, creatine plus phosphocreatine; DD, delayed development; Glx, glutamate plus glutamine; Lac, lactate; ml, *myo*-inositol; NAA, *N*-acetylaspartate; T2r, transverse relaxation expressed as a percentage relative to the TD group; TD, typical development.

by extension, shift glycolytic flux. This methodological factor may have obscured possible disease-related lactate or glutamate and glutamine alterations if they were present. However, distinct patterns of chemical alterations between the ASD-TD and DD-TD pairings provide indirect support that propofol sedation, used in the ASD and DD groups, is not overly confounding.

These compartmental results extend our prior observations of regional neurochemical concentration decreases and T2r prolongation within this group and provide support for a specific abnormality of gray matter early in the clinical course of ASD. These findings may be the result of differences in gray matter connectivity or density. For example, although it remains unclear whether reactive gliosis,<sup>9</sup> minicolumnar pathological abnormalities,<sup>12</sup> serotonergic alterations (as discussed in the article by Gustafsson<sup>44</sup>), or disorganized pyramidal cells<sup>11</sup> are present in cortex at ages 3 to 4 years in ASD, these alterations, if present, could lead to decreased dendritic arborization. Since synaptosomes express high levels of chemicals by MRS<sup>45</sup> and likely have shorter T2 owing to their smaller diameter, such a decrease in dendrite number or density could account for the observed findings. Alternatively, as suggested in our prior work,<sup>23</sup> construction of the brain with larger cellular units would also result in equivalent MRS findings. Although invoking differences in unit size is more speculative, emerging literature describes the host of mechanisms by which developmental pace impacts unit size. Since the signifi-

**Table 3. Statistics and Post Hoc Test Statistics**

Tissue and Metabolite	Statistics						
	Overall		Covariates		Post Hoc Test Statistics		
	F Statistic	P Value	Sex, P Value	Cerebral Volume, P Value	ASD vs TD, P Value	DD vs TD, P Value	ASD vs DD, P Value
Gray matter							
Cho	19.970	<.001	.39	.07	<.001*	.002*	.04*
Cre	2.930	.06	.55	.39	.02*	.19	.48
NAA	3.689	.03	.26	.97	.02*	.59	.09*
ml	6.115	.004	.82	.11	.008*	.94	.008*
Glx	0.837	.44	.90	.35	.53	.20	.34
Lac	0.051	.95	.38	.70	.76	.80	.99
White matter							
Cho	1.179	.32	.23	.59	.14	.24	.98
Cre	1.401	.25	.29	.99	.26	.76	.15
NAA	3.071	.05	.90	.79	.03*	.03*	.58
ml	2.435	.10	.09	.21	.04*	.07*	.76
Glx	1.975	.15	.95	.70	.05	.20	.73
Lac	1.245	.30	.33	.82	.28	.12	.38
T2r in gray matter							
Cho	3.669	.03	.11	.99	.01†	.34	.19
Cre	0.625	.54	.30	.008	.70	.29	.36
NAA	0.682	.51	.17	<.001	.35	.27	.64
T2r in white matter							
Cho	2.005	.14	.04	.32	.13	.06	.38
Cre	1.857	.16	.87	.007	.58	.26	.06
NAA	1.506	.23	.42	.03	.33	.58	.11

Abbreviations: ASD, autism spectrum disorder; Cho, choline-containing compounds; Cre, creatine plus phosphocreatine; DD, delayed development; Glx, glutamate plus glutamine; Lac, lactate; ml, *myo*-inositol; NAA, *N*-acetylaspartate; T2r, transverse relaxation expressed as a percentage relative to the TD group; TD, typical development.

\*Decrease in the statistic (eg, the mean for the ASD group is less than the mean for the TD group).

†Increase in the statistic (eg, the mean for the ASD group is greater than the mean for the TD group).

cant contribution of cerebral volume as a covariate was an unexpected finding of the present study, a brief consideration of scaling seems justified.

A large body of research has investigated brain scaling between species, demonstrating that as cerebral volume increases, the amount of white matter expands with a 4-3 power rule relative to gray matter.<sup>46</sup> This largely reflects the requirement for additional cabling (white matter) necessary to join processing units (gray matter) as cerebral volume expands. For cellular subunits, research on insectivorian species has demonstrated that as the brain enlarges, the neuron-glia ratio and the prevalence of glial subtypes change.<sup>47</sup> Within human brains, it is not clear whether an actual scaling of the cellular subunits occurs in conjunction with increasing brain size. For example, does the number of units scale within a species for a brain that is 10% larger? Or instead, does the size of the units increase with relative conservation of number? In this latter case, it is possible that the observed chemical T2r-cerebral volume relationships reflect the same amount of chemical (eg, NAA) per cellular subunit within a larger intracellular volume as the subunit size increases. The somewhat stronger covariate effect for NAA compared with Cre and Cho might be related to the signal-noise ratio of the measured chemical, the complexity of compartments contributing to the measured signal, and the number of individual resonances contributing to the measured signal.<sup>13</sup> Future work using phosphorous spectroscopy with proton decoupling to measure the separate components of Cho will be helpful to evaluate what underlies this proportionally greater prolongation in ASD.

What remains unclear from the observed relationship between chemical T2r and cerebral volume is whether the association reflects normal scaling factors at ages 3 to 4 years or specific pathological processes in the ASD or DD groups. From the few differences between chemical T2r in premature infants and adults,<sup>48</sup> the latter supposition appears to be more likely. Within the ASD sample, gray matter Cho T2r remained prolonged even after scaling for the effect of cerebral volume, suggesting that this cellular feature may be specifically related to an underlying disease process.

Specific genes that regulate subunit size in mammalian species<sup>49</sup> and may, in part, be mediated through glial signaling and developmental pace<sup>50</sup> have been identified. As proteins such as BCL2 and p53, which are related to apoptosis, have been found to be altered in autism,<sup>51</sup> it seems plausible but clearly speculative that such alterations could impact the size of cell components at this stage in development. A recent animal knock-out study of BCL2 demonstrated severe white matter degeneration that began postnatally,<sup>52</sup> a temporal evolution that has intriguing parallel to many diseases in which early postnatal development appears normal. In other related diseases such as Rett syndrome having symptom overlap with regressive subtypes of autism, alterations in MECP2, a transcriptional regressor, may decrease spine density affecting brain connectivity.<sup>53</sup> Thus, although it is conceptually easier to build a larger brain from more normal-sized units, the potential role of other mechanisms affecting unit size and, by extension, cerebral volume in ASD remains intriguing.

A further point to consider is the potential effect of age or stage of development on the cellular profile in ASD.

Whereas 2 studies in older children<sup>31</sup> and adults<sup>28</sup> have found local regions of increased neurochemistry, we did not observe significantly increased levels of any neurochemical at ages 3 to 4 years in ASD. Although differences in ASD samples (eg, high vs low functioning) and methodological factors (eg, short-echo vs long-echo acquisitions) represent possible methodological confounds, it is notable that the 2 regions identified in those studies (left caudate and right frontal lobe) were not found to be reduced in our initial study,<sup>23</sup> leaving open the possibility that these regions show increasing levels across development. Since the children described in this article are part of an ongoing longitudinal study, it will be important to evaluate this possible progression in future work.

In summary, these results support an altered gray matter chemical environment for children with ASD who are aged 3 to 4 years that is characterized by decreased gray matter chemical concentrations and Cho T2r prolongation. White matter alterations appear to be less specific to autism and may be more related to general developmental pace. Future studies merging genetic subtyping with detailed MRS examination may be helpful to extend and refine the specificity of these observations.

**Submitted for Publication:** April 4, 2005; final revision received December 12, 2005; accepted December 22, 2005.

**Correspondence:** Seth D. Friedman, PhD, Department of Radiology, University of Washington School of Medicine, 1100 NE 45th St NE, Seattle, WA 98105-6099 (seth@u.washington.edu).

**Funding/Support:** This work was funded by program project grant PO1 HD34565 from the National Institute of Child Health and Human Development and the National Institute on Deafness and Communication Disorders.

**Previous Presentation:** This paper was presented at the joint meeting of the Electroencephalography and Clinical Neuroscience Society and the International Society for NeuroImaging in Psychiatry; October 1, 2004; San Diego, Calif.

**Acknowledgment:** We gratefully acknowledge the contributions of the Diagnostic and Statistical Cores of the program project to this study and the administrative support of Marie Domsalla. We also thank magnetic resonance coil engineers Cecil Hayes, PhD, and C. Mark Mathis, BS. Jill Gardner, PhD, provided expertise with image processing. Stephen Provencher, PhD, provided invaluable help in modifying the LCModel software to perform spectroscopic imaging analysis. We extend our sincere thanks to the parents and children who participated in this study.

## REFERENCES

1. Dawson G, Meltzoff AN, Osterling J, Rinaldi J. Neuropsychological correlates of early symptoms of autism. *Child Dev.* 1998;69:1276-1285.
2. Redcay E, Courchesne E. When is the brain enlarged in autism? a meta-analysis of all brain size reports. *Biol Psychiatry.* 2005;58:1-9.
3. Courchesne E, Redcay E, Kennedy DP. The autistic brain: birth through adulthood. *Curr Opin Neurol.* 2004;17:489-496.
4. Herbert MR, Ziegler DA, Deutsch CK, O'Brien LM, Lange N, Bakardjiev A, Hodgson J, Adrien KT, Steele S, Makris N, Kennedy D, Harris GJ, Caviness VS Jr. Dissociations of cerebral cortex, subcortical and cerebral white matter volumes in autistic boys. *Brain.* 2003;126:1182-1192.
5. Sparks BF, Friedman SD, Shaw DW, Aylward EH, Echelard D, Artru AA, Mara-

- villa JN, Giedd JN, Munson J, Dawson G, Dager SR. Brain structural abnormalities in young children with autism spectrum disorder. *Neurology*. 2002;59:184-192.
6. Kanner L. Autistic disturbances of affective contact. *Nerv Child*. 1943;2:217-250.
  7. Palmen SJ, van Engeland H, Hof PR, Schmitz C. Neuropathological findings in autism. *Brain*. 2004;127:2572-2583.
  8. Bauman ML, Kemper TL. Neuroanatomic observations of the brain in autism: a review and future directions. *Int J Dev Neurosci*. 2005;23:183-187.
  9. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol*. 2005;57:67-81.
  10. Miles JH, Takahashi TN, Bagby S, Sahota PK, Vaslov DF, Wang CH, Hillman RE, Farmer JE. Essential vs complex autism: definition of fundamental prognostic subtypes. *Am J Med Genet A*. 2005;135:171-180.
  11. Bailey A, Luthert P, Dean A, Harding B, Janota I, Montgomery M, Rutter M, Lantos P. A clinicopathological study of autism. *Brain*. 1998;121:889-905.
  12. Casanova MF, Buxhoeveden D, Gomez J. Disruption in the inhibitory architecture of the cell minicolumn: implications for autism. *Neuroscientist*. 2003;9:496-507.
  13. Ross B, Michaelis T. Clinical applications of magnetic resonance spectroscopy. *Magn Reson Q*. 1994;10:191-247.
  14. Bluml S, Seymour KJ, Ross BD. Developmental changes in choline- and ethanolamine-containing compounds measured with proton-decoupled (31)P MRS in vivo human brain. *Magn Reson Med*. 1999;42:643-654.
  15. Birken DL, Oldendorf WH. N-acetyl-L-aspartic acid: a literature review of a compound prominent in 1H-NMR spectroscopic studies of brain. *Neurosci Biobehav Rev*. 1989;13:23-31.
  16. Baslow MH. N-acetylaspartate in the vertebrate brain: metabolism and function. *Neurochem Res*. 2003;28:941-953.
  17. Bluml S, McComb JG, Ross BD. Differentiation between cortical atrophy and hydrocephalus using 1H MRS. *Magn Reson Med*. 1997;37:395-403.
  18. Lee JH, Arcinue E, Ross BD. Brief report: organic osmolytes in the brain of an infant with hypernatremia. *N Engl J Med*. 1994;331:439-442.
  19. Manji HK, Lenox RH. Signaling: cellular insights into the pathophysiology of bipolar disorder. *Biol Psychiatry*. 2000;48:518-530.
  20. Gruetter R, Novotny EJ, Boulware SD, Mason GF, Rothman DL, Shulman GI, Prichard JW, Shulman RG. Localized 13C NMR spectroscopy in the human brain of amino acid labeling from D-[1-13C]glucose. *J Neurochem*. 1994;63:1377-1385.
  21. Dager SR, Friedman SD, Parow A, Demopoulos C, Stoll AL, Lyoo IK, Dunner DL, Renshaw PF. Brain metabolic alterations in medication-free patients with bipolar disorder. *Arch Gen Psychiatry*. 2004;61:450-458.
  22. Gadian DG. *NMR and Its Applications to Living Systems*. 2nd ed. New York, NY: Oxford University Press; 1996.
  23. Friedman SD, Shaw DW, Artru AA, Richards TL, Gardner J, Dawson G, Posse S, Dager SR. Regional brain chemical alterations in young children with autism spectrum disorder. *Neurology*. 2003;60:100-107.
  24. Hashimoto T, Tayama M, Miyazaki M, Yoneda Y, Yoshimoto T, Harada M, Miyoshi H, Tanouchi M, Kuroda Y. Differences in brain metabolites between patients with autism and mental retardation as detected by in vivo localized proton magnetic resonance spectroscopy. *J Child Neurol*. 1997;12:91-96.
  25. Otsuka H, Harada M, Mori K, Hisaoka S, Nishitani H. Brain metabolites in the hippocampus-amygdala region and cerebellum in autism: an 1H-MR spectroscopy study. *Neuroradiology*. 1999;41:517-519.
  26. Mori K, Hashimoto T, Harada M, Yoneda Y, Shimakawa S, Fujii E, Yamaue T, Miyazaki M, Saijo T, Kuroda Y. Proton magnetic resonance spectroscopy of the autistic brain [in Japanese]. *No To Hattatsu*. 2001;33:329-335.
  27. Hisaoka S, Harada M, Nishitani H, Mori K. Regional magnetic resonance spectroscopy of the brain in autistic individuals. *Neuroradiology*. 2001;43:496-498.
  28. Murphy DG, Critchley HD, Schmitz N, McAlonan G, Van Amelsvoort T, Robertson D, Daly E, Rowe A, Russell A, Simmons A, Murphy KC, Howlin P. Asperger syndrome: a proton magnetic resonance spectroscopy study of brain. *Arch Gen Psychiatry*. 2002;59:885-891.
  29. Fayed N, Modrego PJ. Comparative study of cerebral white matter in autism and attention-deficit/hyperactivity disorder by means of magnetic resonance spectroscopy. *Acad Radiol*. 2005;12:566-569.
  30. Posse S, Dager SR, Richards TL, Yuan C, Ogg R, Artru AA, Muller-Gartner HW, Hayes C. In vivo measurement of regional brain metabolic response to hyperventilation using magnetic resonance: proton echo planar spectroscopic imaging (PEPSI). *Magn Reson Med*. 1997;37:858-865.
  31. Levitt JG, O'Neill J, Blanton RE, Smalley S, Fadale D, McCracken JT, Guthrie D, Toga AW, Alger JR. Proton magnetic resonance spectroscopic imaging of the brain in childhood autism. *Biol Psychiatry*. 2003;54:1355-1366.
  32. Doyle TJ, Bedell BJ, Narayana PA. Relative concentrations of proton MR visible neurochemicals in gray and white matter in human brain. *Magn Reson Med*. 1995;33:755-759.
  33. Pfefferbaum A, Adalsteinsson E, Spielman D, Sullivan EV, Lim KO. In vivo spectroscopic quantification of the N-acetyl moiety, creatine, and choline from large volumes of brain gray and white matter: effects of normal aging. *Magn Reson Med*. 1999;41:276-284.
  34. Lord C, Rutter M, Le Couteur A. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Dev Disord*. 1994;24:659-685.
  35. Lord C, Risi S, Lambrecht L, Cook EH Jr, Leventhal BL, DiLavore PC, Pickles A, Rutter M. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev Disord*. 2000;30:205-223.
  36. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*. Washington, DC: American Psychiatric Association; 1994.
  37. Mullen E. *Mullen Scales of Early Learning: AGS Edition*. Circle Pines, Minn: American Guidance Service; 1995.
  38. Sparrow SS, Balla DA, Cicchetti DV. *Vineland Adaptive Behavior Scales: Interview Edition, Survey Form Manual*. Circle Pines, Minn: American Guidance Service; 1984.
  39. Sled JG, Zijdenbos AP, Evans AC. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans Med Imaging*. 1998;17:87-97.
  40. Bouman C, Shapiro M. A multiscale random field model for Bayesian image segmentation. *IEEE Trans Image Process*. 1994;3:162-177.
  41. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med*. 1993;30:672-679.
  42. Chugani DC, Sundram BS, Behen M, Lee ML, Moore GJ. Evidence of altered energy metabolism in autistic children. *Prog Neuropsychopharmacol Biol Psychiatry*. 1999;23:635-641.
  43. Artru AA. Propofol combined with halothane or with fentanyl/halothane does not alter the rate of CSF formation or resistance to reabsorption of CSF in rabbits. *J Neurosurg Anesthesiol*. 1993;5:250-257.
  44. Gustafsson L. Comment on "Disruption in the inhibitory architecture of the cell minicolumn: implications for autism." *Neuroscientist*. 2004;10:189-191.
  45. Petroff OA, Pleban LA, Spencer DD. Symbiosis between in vivo and in vitro NMR spectroscopy: the creatine, N-acetylaspartate, glutamate, and GABA content of the epileptic human brain. *Magn Reson Imaging*. 1995;13:1197-1211.
  46. Zhang K, Sejnowski TJ. A universal scaling law between gray matter and white matter of cerebral cortex. *Proc Natl Acad Sci U S A*. 2000;97:5621-5626.
  47. Stolzenburg JU, Reichenbach A, Neumann M. Size and density of glial and neuronal cells within the cerebral neocortex of various insectivorous species. *Glia*. 1989;2:78-84.
  48. Kugel H, Roth B, Pillekamp F, Kruger K, Schulte O, von Gontard A, Benz-Bohm G. Proton spectroscopic metabolite signal relaxation times in preterm infants: a prerequisite for quantitative spectroscopy in infant brain. *J Magn Reson Imaging*. 2003;17:634-640.
  49. Dolznic H, Grebien F, Sauer T, Beug H, Mullner EW. Evidence for a size-sensing mechanism in animal cells. *Nat Cell Biol*. 2004;6:899-905.
  50. Ullian EM, Sapperstein SK, Christopherson KS, Barres BA. Control of synapse number by glia. *Science*. 2001;291:657-661.
  51. Fatemi SH, Halt AR. Altered levels of Bcl2 and p53 proteins in parietal cortex reflect deranged apoptotic regulation in autism. *Synapse*. 2001;42:281-284.
  52. Zhang J, Chen YB, Hardwick JM, Miller MI, Plachez C, Richards LJ, Yarowsky P, van Zijl P, Mori S. Magnetic resonance diffusion tensor microimaging reveals a role for Bcl-x in brain development and homeostasis. *J Neurosci*. 2005;25:1881-1888.
  53. Glaze DG. Rett syndrome: of girls and mice: lessons for regression in autism. *Ment Retard Dev Disabil Res Rev*. 2004;10:154-158.