Atypical Developmental Patterns of Brain Chemistry in Children With Autism Spectrum Disorder

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**IMPORTANCE** Autism spectrum disorder (ASD) is a neurodevelopmental disorder with symptoms emerging during early childhood. The pathophysiology underlying the disorder remains incompletely understood.

**OBJECTIVE** To examine cross-sectional and longitudinal patterns of brain chemical concentrations in children with ASD or idiopathic developmental delay (DD) from 3 different age points, beginning early in the clinical course.

**DESIGN** Proton magnetic resonance spectroscopic imaging data were acquired longitudinally for children with ASD or DD, and primarily cross-sectionally for children with typical development (TD), at 3 to 4, 6 to 7, and 9 to 10 years of age.

**SETTING** Recruitment, diagnostic assessments, and magnetic resonance imaging were performed at the University of Washington in Seattle.

**PARTICIPANTS** Seventy-three children (45 with ASD, 14 with DD, and 14 with TD) at 3 to 4 years of age; 69 children (35 with ASD, 14 with DD, and 20 with TD) at 6 to 7 years of age; and 77 children (29 with ASD, 15 with DD, and 33 with TD) at 9 to 10 years of age.

**MAIN OUTCOMES AND MEASURES** Concentrations of N-acetylaspartate (NAA), choline (Cho), creatine (Cr), myo-inositol (mI), and glutamine plus glutamate (Glx) in cerebral gray matter (GM) and white matter (WM) at 3 to 4, 6 to 7, and 9 to 10 years of age, and calculation of rates of change of these chemicals between 3 and 10 years of age.

**RESULTS** At 3 to 4 years of age, the ASD group exhibited lower NAA, Cho, and Cr concentrations than did the TD group in both GM and WM, alterations that largely were not observed at 9 to 10 years of age. The DD group exhibited reduced GM and WM NAA concentrations at 3 to 4 years of age; GM NAA concentrations remained reduced at 9 to 10 years of age compared with the TD group. There were distinct differences between the ASD and DD groups in the rates of GM NAA, Cho, and Cr changes between 3 and 10 years of age.

**CONCLUSIONS AND RELEVANCE** The GM chemical changes between 3 and 10 years of age differentiated the children with ASD from those with DD. Most notably, a dynamic reversal of GM NAA reductions was observed in the children with ASD. By contrast, persistent GM NAA reductions in the children with DD suggest a different, more static, underlying developmental process.
Autism spectrum disorder (ASD) is characterized by deficits in social communication, repetitive behaviors, and restricted interests, with initial symptoms expressed during early childhood. Although genetic and environmental factors are known to play a role in the etiology of the disorder, the underlying pathophysiology remains elusive. We, and others, have reported cerebral enlargement in preschool-aged children with ASD.\(^1\)\(^-\)\(^3\) Enlargement is not uniform but varies between brain regions, as well as between the gray matter (GM) and white matter (WM) compartments.\(^2\)\(^-\)\(^4\) Head circumference trajectories and findings from the few available longitudinal imaging studies to date indicate that brain enlargement in children with ASD is dynamic during the early course of development, with evidence for normal volume at birth followed by progressive enlargement that plateaus during the early school years.\(^1\)\(^,\)\(^5\) Evidence for cerebral enlargement during later childhood or in adulthood is less conclusive,\(^6\)\(^,\)\(^7\) suggesting that a dynamic pathophysiological process underlies brain development in ASD.

Proton magnetic resonance spectroscopy ('H-MRS) allows for the measurement of brain tissue-based chemicals that can help to shed light on processes at the cellular level.\(^2\) Chemicals that can be measured with 'H-MRS at 1.5 T include N-acetylaspartate (NAA), choline (Cho), creatine (Cr), myoinositol (ml), lactate, and the combined visible signal from glutamate and glutamine (Glx). To date, 'H-MRS studies of ASD have most often used single-voxel techniques that allow for sampling of only one large region of the brain at a time, with many of these studies assessing high functioning adults or pooling together subjects across a wide range of ages.\(^8\) Proton magnetic resonance spectroscopic imaging ('H-MRSI)\(^7\) has been used by a handful of researchers to study ASD.\(^9\)\^-\(^15\) Although results from 'H-MRSI studies have been variable, reduced NAA or NAA/Cre has been the most consistent finding, and more consistently observed in younger populations.\(^8\) A recent meta-analysis of 22 'H-MRS studies similarly reported decreased NAA in ASD, with greater differences in childhood than adulthood.\(^16\)

Using an 'H-MRSI echo-planar technique\(^7\) to evaluate for both regional anatomical differences\(^8\) and differences between GM and WM tissue compartments,\(^19\) we previously reported abnormal brain chemical findings in children with ASD at 3 to 4 years of age. In the present study, we report longitudinal 'H-MRSI findings between 3 and 10 years of age for the same cohort of children with ASD or idiopathic developmental delay (DD) without autism in comparison with a predominantly cross-sectional sample of children with typical development (TD). Our study builds on a recent report based on longitudinal data from this same cohort that used a different (long-echo) 'H-MRSI pulse sequence (with an echo time [TE] of 272 milliseconds) to evaluate for brain metabolic and structural evidence of mitochondrial dysfunction in ASD.\(^18\) The present study used a short-echo 'H-MRSI pulse sequence with acquisition parameters optimized for accurate measurement of chemicals that cannot be accurately measured at the longer echo time.\(^7\) For this report, we reanalyzed the previously reported data on 3- to 4-year-old children\(^9\)\(^-\)\(^11\) and the data acquired at subsequent ages using a recently developed 'H-MRSI analytic approach that utilized the large number of voxels acquired to improve the reliability of chemical measurements and the coregistration between tissue compartments.\(^19\) Our hypothesis, based on the available literature,\(^8\) was that children with ASD would exhibit a pattern of NAA changes, and possibly other measurable chemical changes, that would distinguish them from the children with TD or DD. We present longitudinal 'H-MRSI findings for NAA, Cho, Cr, ml, and Glx concentrations for the GM and WM tissue compartments at 3 to 4, 6 to 7, and 9 to 10 years of age.

### Methods

#### Participants

We studied children with ASD diagnosed as young as was clinically feasible at the time our study was initiated, and we focused on narrow age ranges at initial and longitudinal follow-up assessments. In total, 73 children (45 with ASD, 14 with DD, and 14 with TD) at 3 to 4 years of age, 69 children (35 with ASD, 14 with DD, and 20 with TD) at 6 to 7 years of age, and 77 children (29 with ASD, 15 with DD, and 33 with TD) at 9 to 10 years of age were scanned. A subset of these children (1 with DD and 4 with TD at 3 to 4 years of age; 4 with ASD and 2 with TD at 6 to 7 years of age; and 3 with DD and 4 with TD at 9 to 10 years of age) did not have usable 'H-MRSI data. Demographic and clinical information for children with usable data are shown in Table 1. Participant drop-out resulted in the assessment of some children in these diagnostic groups at only 1 or 2 time points. Usable data from all 3 age groups were available for 17 children with ASD, 7 children with DD, and 1 child with TD. Usable data for 2 time points were available from an additional 18 children with ASD, 5 children with DD, and 6 children with TD. Usable data at 1 time point were available for 18 children with ASD, 8 children with DD, and 42 children with TD. Additional children from a larger pool of subjects followed behaviorally from 3 to 4 years were included at the later ages to address subject attrition. Data from a total of 122 unique children across all 3 age groups (53 with ASD, 20 with DD, and 49 with TD) were included in these analyses.

Children were recruited from local parent advocacy groups, the University of Washington Infant and Child Subject Pool, public schools, clinics, hospitals, and the Department of Developmental Disabilities. Exclusion criteria included identifiable genetic abnormalities (including fragile X syndrome, Norrie disease, neurofibromatosis, tuberous sclerosis, and phenylketonuria), cerebral vascular disease, severe sensory or motor impairments, significant pulmonary disease, unstable cardiovascular status, major physical abnormalities, medically significant perinatal difficulties, documented prenatal or postnatal head trauma, and implanted medical prostheses or other devices incompatible with magnetic resonance imaging (MRI) scanning. A history of seizures was exclusionary at enrollment; however, 1 child with ASD had seizures when studied at 6 to 7 years of age, and an additional 2 children with ASD had seizures at 9 to 10 years of age. Written parental/guardian informed consent to participate in the study, approved by the...
University of Washington Human Subjects Division, was obtained for each participant.

A diagnosis of ASD was based on the Autism Diagnostic Interview–Revised,\textsuperscript{20} the Autism Diagnostic Observation Schedule–Generic,\textsuperscript{21} and an assessment by an experienced clinician using DSM-IV diagnostic criteria.\textsuperscript{22} The ASD sample of participants included children with a range of intellectual abilities. A diagnosis of DD was based on a review of the medical records to exclude perinatal complications, fetal alcohol syndrome, or identifiable genetic disorders associated with ASD and scores consistent with DD on the Mullen Scales of Early Learning\textsuperscript{23} and the Vineland Adaptive Behavior Scales.\textsuperscript{24} Children in the DD group did not show symptoms of autism based on the Autism Diagnostic Observation Schedule–Generic. Children with ASD and children with DD exhibited significant developmental delay at study enrollment and were evenly matched with regard to the Mullen Composite Age Equivalent score\textsuperscript{25} (mean [SD] score, 25.9 [9.2] vs 25.5 [8.1] months). Children in the TD group scored within 1 SD of the average on the Vineland Adaptive Behavior Scales and had no history of language, social, motor, or cognitive delay; speech therapy; psychiatric disturbances; or learning problems. Diagnoses for children in the ASD and DD groups were determined prior to the first MRI scan for each child and confirmed at subsequent time points using equivalent age-appropriate diagnostic instruments. A subset of children from the ASD and DD groups were treated with psychotropic medications, as detailed in Table 1.

### Table 1. Demographics and Medication Status of Participants

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Children, No.</th>
<th>Sex, No.</th>
<th>Age, Mean (SD), y</th>
<th>Participant(s) on Medication, No.</th>
<th>Medication(s) Prescribed</th>
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<tr>
<td>3-4 y</td>
<td></td>
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<tr>
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<td>45</td>
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<td>6</td>
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<td>10</td>
<td>8</td>
<td>2</td>
<td>3.8 (0.43)</td>
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<td>6-7 y</td>
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<tr>
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<td>24</td>
<td>7</td>
<td>6.6 (0.39)</td>
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<tr>
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<tr>
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<tr>
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<td>29</td>
<td>25</td>
<td>4</td>
<td>9.6 (0.39)</td>
<td></td>
</tr>
</tbody>
</table>

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

Study Procedures

Children in the ASD and DD groups were scanned during continuous intravenous infusion of propofol at 180 to 220 μg·kg⁻¹·min⁻¹, with the exception of 1 child with ASD at 6 years of age and 2 children with ASD at 9 years of age who were scanned without sedation. Physiological parameters were monitored continuously during sedation by an experienced anesthesiologist (A.A.A.). Children in the TD group were observed late at night during natural sleep. Sedation with diphenhydramine hydrochloride (25 mg orally) administered by the parents was used for 8 children with TD who were 3 to 4 years of age.

All data were acquired on the same 1.5 T GE Signa Horizon whole-body MRI scanner with Genesis operating software (version 5.8) located at the University of Washington Diagnostic Imaging Sciences Center. A custom receive-only linear birdcage radiofrequency head coil with a built-in head holder to immobilize the head, developed at the University of Washington, was used for data collection. Axial proton density and T2-weighted images covering the entire sampled volume were acquired, with TE = 13/91 milliseconds, a repetition time of 2000 milliseconds, a 22-cm field of view, a 256 × 160 matrix, and a 2.5-mm slice thickness. Proton echo-planar spectroscopic imaging (PEPSI)\textsuperscript{17} was used for rapid acquisition of 1H-MRSI data. Unsuppressed water (TE = 20 milliseconds) and water-suppressed (TE = 272/20 milliseconds) PEPSI scans (with a repetition time of 2000 milliseconds, a 32 × 32 spatial matrix, a nominal voxel size of 1 cm³, a 22-cm field of view, and a 20-mm slice thickness) were acquired either from 2 contiguous sections—with the first section centered on the anterior commissure, and the second section through the basal ganglia (for the age groups of 3-4 and 6-7 years of age)—or from a single axial section through the basal ganglia (for the age group of 9-10 years of age). Short-echo (TE = 20 milliseconds) metabolite data are included in the present analysis, with the water PEPSI scans used for chemical quantification.\textsuperscript{19}

Structural Image Segmentation

For each participant, images with enhanced GM and WM contrast were produced by summing the proton density and T2-weighted MRI. Images with enhanced tissue/cerebrospinal fluid contrast were then created by subtracting the T2-weighted images from the proton density images. These contrast-enhanced images were corrected for radiofrequency inhomogeneity using homomorphic filtering.\textsuperscript{25} The resulting images were classified using a k-means algorithm to produce binary
GM, WM, and cerebrospinal fluid images and then averaged together.\(^26\) These images were filtered to produce final images with a point-spread function approximating that of the spectroscopic images.\(^{11}\)

**1H-MRSI Analyses**

Spectroscopic data were reconstructed and preprocessed with the LCModel software package (version 6.2).\(^27\) For each participant, phased and peak-aligned free induction decay (FID) signals output from initial LCModel processing were averaged for all voxels in each brain compartment to produce a single metabolite FID signal and a single water FID signal.\(^{19}\) In cases in which 2 slabs were acquired, all FID signals in the compartment from both slabs were averaged. Spectra from individual voxels were excluded if the full width at half maximum of the NAA peak exceeded 0.2 ppm or if the Cramer-Rao lower bound for Cho, Cr, or NAA exceeded a value of 20. The averaged FID signal was fitted with LCModel, and chemical concentrations were computed using methods detailed in the LCModel User’s Manual (http://s-provencher.com/pages/lcm-manual.shtml). The NAA signal measured in our study includes contributions from \(N\)-acetylaspartate and \(N\)-acetylaspartylglutamate, the Cho signal includes contributions from phosphorycholine and glycerophosphorylcholine, the Cr signal includes contributions from creatine and phosphocreatine, and the Glx signal includes contributions from glutamate and glutamine.

Chemical concentrations were calculated for GM and WM brain tissue compartments based on the segmentation images. Specifically, individual PEPSI voxels containing more than 60% GM were included in a GM compartment, and voxels containing more than 60% WM were included in a WM compartment.\(^{18}\) Example GM spectra and LCModel fits for a child with ASD who was scanned longitudinally at 3, 6, and 9 years of age are shown in Figure 1.

**Statistical Analyses**

To investigate diagnostic group differences, 1-way analysis of variance was used to test for significant differences in chemical concentrations among the 3 diagnostic groups for each compartment and age group. Sex was also assessed as a covariate. For analysis of variance reaching significance, post hoc pairwise testing was performed using the Bonferroni adjustment and a 2-tailed significance level of .05. All cross-sectional statistical analyses were performed with PASW Statistics 18.0.

Linear mixed-effects modeling was used to examine diagnostic group differences between the ASD and DD groups in the trajectories of neurochemical concentration changes over time using the “nlme” package in R with a restricted maximum likelihood estimation (Pinheiro et al\(^\text{28}\) linear and non-linear mixed-effects models; R package version 3.1-104). To be conservative, the TD group was not included in this assessment because it included mostly participants with only cross-sectional data. Children in the ASD and DD groups with usable data for only 1 time point were included in the multilevel models, but their data were not used in the estimation of slope parameters.\(^{29}\) The intercept was centered at 9.5 years of age (the mean age of the children at 9-10 years of age). Both the intercept and the slope (or age effect) were treated as random effects, allowing them to vary across participants.

**Results**

**Cross-Sectional Findings**

Cross-sectional mean (SD) chemical measures and summaries of statistical analyses for each brain compartment and age group by diagnostic group are shown in Table 2. Sex did not affect diagnostic group relationships and did not account for a significant portion of the variance, with the sole exception of GM Cho at 9 to 10 years of age \((P = .05)\).
At 3 to 4 years of age, diagnostic group NAA differences were observed in both GM ($F_{2,65} = 7.15$, $P = .002$) and WM ($F_{2,65} = 10.54$, $P < .001$); relative to the TD group, both the ASD ($−10.1\%$, $P = .001$; $−9.4\%$, $P < .001$) and DD ($−8.2\%$, $P = .04$; $−7.6\%$, $P = .008$) groups demonstrated lower NAA levels in GM and WM, respectively. At 6 to 7 years of age, group differences in NAA were not found for either compartment ($P > .12$). Findings at 9 to 10 years of age revealed group NAA differences in GM ($F_{2,67} = 3.33$, $P = .04$) and WM ($F_{2,65} = 3.22$, $P = .046$), with lower GM NAA levels in the DD group relative to the TD group ($−9.1\%$, $P = .04$) and lower WM NAA levels in the ASD group relative to the TD group ($−5.1\%$, $P = .04$).

### Choline

Choline differences in GM at 3 to 4 years of age ($F_{2,65} = 12.88$, $P < .001$) reflected lower Cho levels for both the ASD and DD groups in comparison with the TD group ($−20.8\%$, $P < .001$; $−16.6\%$, $P < .001$).
Group differences at 3 to 4 years of age were found for Cho levels in WM at 3 to 4 years of age ($F_{2,65} = 4.07, P < .02$) reflected lower Cho levels in the ASD group than in the TD group ($−9.2%, P = .02$). At 6 to 7 years of age, group differences in Cho levels in GM ($F_{2,60} = 5.76, P = .005$) reflected lower Cho levels in both the ASD group ($−10.1%, P = .001$) and the DD group ($−18.8%, P = .005$) groups. At 6 to 7 years of age, WM Cho differences ($F_{2,60} = 3.22, P = .047$) reflected a trend of increasing Cho levels for the ASD group compared with the TD group ($6.3%, P = .07$). No significant group differences in Cr levels were found for either compartment at 9 to 10 years of age. At 9 to 10 years of age, differences in Cho levels in GM were greater in the DD group ($12.0%, P = .001$) and WM ($18.8%, P = .005$) groups. At 9 to 10 years of age, there were group differences in WM Glx concentrations ($F_{2,60} = 3.82, P = .02$), with a trend toward lower Glx levels in the DD group than in the ASD group ($−18.8%, P = .06$). At 9 to 10 years of age, there were no group differences for either compartment (all $P > .05$).

Longitudinal Findings
Slopes of chemical changes in units of millimolar per month between the 3- to 4-year-old and 9- to 10-year-old age groups for the ASD and DD groups (based on the mixed-effects modeling), along with statistical differences between the 2 groups, are shown in Table 3. Scatterplots of individual brain chemical concentrations for all participants of each diagnostic group, along with the linear mixed-effects model trajectory over time for the ASD and DD groups, are shown for the GM compartment in Figure 2 and for the WM compartment in Figure 3.

Across the age groups, there was a substantially greater increase in GM NAA levels for the ASD group than for the DD group ($t = −2.56, P = .013$). There was no difference in the slopes of WM NAA changes between groups ($P = .18$).

Choline levels in GM and WM decreased for both groups; the rate of decrease in GM was steeper for the DD group than for the ASD group ($t = −2.06, P = .04$), whereas differences in the slope of the decreases in WM were at a trend level of significance ($t = −1.68, P = .10$). Creatine concentrations in GM decreased only for the DD group, whereas Cr concentrations in WM decreased for both the ASD and DD groups; the slope of change in Cr concentrations in GM was greater in the DD group than in the ASD group ($t = −2.35, P = .02$), whereas slope differences in WM were at a trend level of significance ($t = −1.73, P = .09$), with a more negative slope observed for the DD group.

Concentrations of ml decreased in both the GM and WM compartments for the 2 diagnostic groups. There was a trend difference in the rate of decrease in ml levels for the ASD group compared with the DD group in GM ($t = −1.70, P = .09$), as well as in WM ($t = −1.79, P = .08$), with a steeper decrease in ml lev-
els across time for the DD group in both compartments. There were no slope differences in GM Glx changes between groups ($P = .50$), but there were trend differences in the slopes of WM Glx changes ($t = -1.75, P = .08$), with the DD group exhibiting a steeper rate of decrease than the ASD group.

**Discussion**

Results from our study demonstrate dynamic $^1$H-MRSI chemical changes in the brains of young children with ASD during...
childhood development that are distinct from the patterns observed in children with DD. In particular, reduced GM NAA levels were observed in both the ASD and DD groups at 3 to 4 years of age, but NAA levels rapidly increased for the ASD group between 3 and 10 years of age to the levels found in the TD group, whereas the DD group exhibited a minimal increase in NAA levels over this time period, and the levels remained reduced compared with the levels in the TD group at 9 to 10 years of age. There were also slope differences for GM Cho and Cr changes between the ASD and DD groups.

Figure 3. White Matter Chemical Concentrations

White matter chemical concentrations by diagnostic group plotted vs age. Individual data points represent measurements of chemical concentrations for individual participants. The yellow and blue lines represent the linear mixed-effects model results for the autism spectrum disorder (ASD) and developmental delay (DD) groups, respectively. The dashed gray line, which represents a simple linear fit of the cross-sectional data points for the typical development (TD) group, is shown as a point of reference but is not directly comparable to the longitudinal findings of the other 2 groups. Cho indicates choline; Cr, creatine; Glx, glutamine plus glutamate; mI, myo-inositol; and NAA, N-acetylaspartate.
Substantial evidence points to the involvement of NAA in the normal development and maintenance of the central nervous system. N-acetylaspartate and NAAG, synthesized through the addition of glutamate to NAA, appear to play critical roles in energy regulation, myelination, cellular signaling, regulation of cellular osmosis, neurotransmission, and as storage pools for glutamate and aspartate in the brain. N-acetylaspartate and NAAG transport between neurons, oligodendrocytes, microglia, and astrocytes has been proposed to comprise a cellular signaling system that influences the migration and functional modulation of these cells during brain development and plays a role in regulating synaptic connections. The 1H-MRS signal from Cho primarily reflects unbound Cho-containing molecules that are components of the myelin sheath. In the typically developing brain, decreases in Cho reflect an incorporation of these macromolecules into the myelin, rendering them largely invisible to MRS. Although our findings of decreased NAA and Cho concentration at 3 to 4 years of age in the ASD group could potentially reflect alterations in neuronal myelination at this stage of development, reports of childhood brain development from the literature suggest that the bulk of myelination has been completed by this age, which suggests alternative but unknown mechanistic explanations.

The pattern of chemical changes in the ASD group during the time period examined in our study is comparable to brain chemical changes observed in other disorders, such as multiple sclerosis, epilepsy, and traumatic brain injury, in which the NAA level is reduced at the time of onset or insult, with reversals or increases in NAA concentration often observed during periods of remission, with successful treatment or with recovery. A return to homeostasis after a disruptive event during earlier development is consistent with theories of early brain inflammatory processes, as yet unproven, as a causal mechanism for cerebral enlargement observed in children with ASD during the preschool years. The rapid increase in GM NAA levels in the children with ASD, in contrast to the children with DD, would further suggest a dynamic and recoverable disease process that can result in other downstream effects but does not, in itself, persist over time. Gray matter NAA levels in the children with DD did not appreciably increase and remained reduced compared with GM NAA levels in children with TD. This notion of recovery from an earlier alteration in normal brain development in ASD is, thus, consistent with findings of functional abnormalities in connectivity later in life in ASD, as even a relatively brief period of abnormal signaling between glial cells and neurons during early development would likely have a lasting effect on the pattern of brain connectivity, which patterns are in large part developed through this early cell signaling. These results also are consistent with the paucity of findings of reduced NAA levels in adults with ASD. The results from our study are further in keeping with findings of gene alterations in ASD specifically associated with a network of proteins located at the pre-synaptic terminals and postsynaptic densities (neurexin/neuroligin/SHANK proteins) that may result in altered synaptogenesis or an improper organization and maintenance of synaptic connections during early development.

Early alterations in brain chemical concentrations, with a dynamic reversal over time, are also consistent with behavioral studies. The symptoms of, and the impairment associated with, ASD remain clinically significant throughout a person’s life span; however, an improvement in ASD-specific symptoms may occur over the course of childhood through adulthood. At the same time that ASD-specific symptoms appear to be lessening over time, evidence suggests that young adults are at risk for increased social isolation and very significant levels of associated conditions, such as seizures. Reductions in NAA levels have been associated with mitochondrial hypometabolism, although generally in association with concurrent evidence for bioenergetic dysregulation, particularly elevated lactate levels. We previously reported that this same ASD cohort did not have elevations in lactate levels or MRI abnormalities characteristic of mitochondrial dysfunction at any of the 3 time points. Moreover, mitochondrial dysfunction is an unlikely mechanism to account for the overall pattern of altered cross-sectional chemical concentrations and changes over the developmental time course observed in our study. The Glx levels were also not elevated, as would be expected with mitochondrial dysfunction. Observations of lower Glx levels may reflect reduced glutamate levels, but a further investigation of this finding awaits the application of newer acquisition techniques at higher-field strength to resolve the individual components of the Glx signal.

The mI levels in GM and WM were not found to significantly differ between the ASD and TD groups at any time point, whereas the mI levels in WM were higher in the DD group than in the ASD group at 3 to 4 years of age and were higher in the DD group than in both the ASD and TD groups at 6 to 7 years of age. There also were trend differences in the slopes of mI change for both GM and WM, with a steeper rate of decrease observed for the DD group between 3 and 10 years of age. Increased levels of mI in the brain have been similarly observed in Down syndrome. Myo-inositol is an important regulator of brain osmotic balance and is a precursor for the phosphatidylinositol second messenger system. Through this second messenger system, it affects regional calcium fluctuations that help to mediate complex cellular processes, such as cell migration, during typical development. A putative marker for astrocytes, high levels of mI can also reflect astrocyte overproliferation. Because astrocytes also have relatively high concentrations of Cr, it is perhaps noteworthy that the DD group also exhibited elevated Cr levels in WM at 3 to 4 years of age compared with the ASD group.

Our study utilized an improved 1H-MRSI analytic approach to reanalyze the short-echo 1H-MRSI data previously reported for the same sample of 3- to 4-year-olds, in order to allow comparisons with data acquired at the later time points. The GM findings at the 3- to 4-year-old time point did not substantively change from our earlier report for this sample, although diagnostic group differences in WM chemical concentrations between the ASD and DD groups in our earlier report using regression methods reached significance with the use of the new analytic approach. A limitation of our study...
is the small number of children with TD (n = 10) with usable data at 3 to 4 years of age and the primarily cross-sectional composition of the TD group across the age groups. Furthermore, a subset of participants with ASD or DD were treated with psychotropic medications, primarily at later ages, as detailed in Table 1. It also should be noted that, by averaging data across GM and WM regions, we assume uniform metabolite distributions within these compartments. Although a regional analysis of the longitudinal data is beyond the scope of our study, an analysis of the chemical changes by anatomical brain region will be the focus of follow-up reports.

Our study design used propofol for scanning the children with ASD or DD. Although essential to the successful completion of this research, propofol represents a potential methodological confounding factor when comparing the results from the ASD and DD groups with the results from the TD group, for which propofol was not used. The metabolic effects of propofol have been well characterized, and it has been found to reduce brain activation in a uniform manner, in contrast to other dissociative anesthetics. Furthermore, the vital signs of all the children with ASD or DD were monitored in real time during 1H-MRSI acquisition, and they all showed stable blood pressure, heart rate, end-tidal carbon dioxide, tidal volume, and hemoglobin oxygen saturation, and no compartmental or global increases in lactate levels in the brain were found. Because the 1H-MRS peak height can increase and the 1H-MRS peak line width can decrease in response to neuronal activation, as has been demonstrated at high fields, it is conceivable that decreased neuronal activation resulting from propofol administration could have the opposite effects on peak characteristics. However, because metabolite data were referenced to brain water content, which also changed in the aforementioned study, this mechanism seems an unlikely bias. A recent study that evaluated the effects of propofol on 1H-MRS brain metabolite measurements found variable regional reductions at anesthetic doses, but for sedative doses, as was used in our study, NAA concentrations were not altered in any of the measured regions, although Cho concentrations were found to be reduced in 1 of 5 brain regions measured (ie, the thalamus), and glutamate concentrations were reduced in 3 regions (the thalamus and the motor and sensory cortices). This potential confounding factor would not influence our trajectory findings between the ASD and DD groups, but we advise caution in interpreting cross-sectional Cho and Glx findings in relationship to the TD group.

We previously reported brain lactate findings from an analysis of long echo-time (TE = 272 milliseconds) 1H-MRSI data acquired from the same sample of participants as assessed in the present study. A long echo time was chosen for that study in order to optimize the measurement of lactate and to reduce the overlapping signal from lipids and macromolecules that occur at shorter echo times. In prior work, which characterized differences in chemical relaxation measures between children with ASD and children with TD or DD, we found that longer echo times were not suitable for accurately measuring NAA, Cho, Cr, mI, and Glx levels because there are metabolite relaxation differences between diagnostic groups that could influence the results. The short echo time used for the present study minimized relaxation effects and provided a more accurate assessment of these chemical concentrations.

In conclusion, the longitudinal application of 1H-MRSI to systematically evaluate brain chemistry in young children with ASD revealed age-related alterations between 3 and 10 years of age that were distinct from those found in the children with DD. Developmental patterns of chemical changes in the ASD group, most notably a dynamic reversal of GM NAA reductions, were not similarly observed in the longitudinal assessment of the DD group, in which persistent lower GM NAA levels at 9 to 10 years of age were observed in comparison with the TD group. The results from our study suggest that a dynamic brain developmental process underlies ASD, whereas the children with DD exhibited a different, more static developmental pattern of brain chemical changes. The brain chemical alterations observed in the children with ASD at 3 to 4 years of age likely reflect a process that begins at an earlier stage of development. Future studies at even younger ages may help to elucidate the timing and underlying pathophysiology of this brain developmental process.

REFERENCES
11. Friedman SD, Shaw DW, Artru AA, Dawson G, Petropoulos H, Dager SR. Gray and white matter...