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A proton spectroscopy study of white matter in children with autism

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ABSTRACT

White matter abnormalities have been described in autism spectrum disorder (ASD) with mounting evidence implicating these alterations in the pathophysiology of the aberrant connectivity reported in this disorder. The goal of this investigation is to further examine white matter structure in ASD using proton magnetic resonance spectroscopy (^{1}H MRS). Multi-voxel, short echo-time *in vivo* ^{1}H MRS data were collected from 17 male children with ASD and 17 healthy age- and gender-matched controls. Key ^{1}H MRS metabolite ratios relative to phosphocreatine plus creatine were obtained from four different right and left white matter regions. Significantly lower N-acetylaspartate/creatine ratios were found in the anterior white matter regions of the ASD group when compared to controls. These findings reflect impairment in neuroaxonal white matter tissue and shed light on the neurobiologic underpinnings of white matter abnormalities in ASD by implicating an alteration in myelin and/or axonal development in this disorder.

1. Introduction

Autism spectrum disorder (ASD) is characterized by impairments in social interaction, communication, and stereotyped and/or repetitive behaviors (APA, 2000). ASD represents a set of etiologically heterogeneous neurodevelopmental disorders but appear to share common biologic abnormalities implicating alterations in neural connectivity, involving short- and long-distance connections potentially contributing to the development of some symptoms of ASD (Courchesne and Pierce, 2005; Just et al., 2007). The integrity of several elements including neuronal bodies and axons as well as myelin sheath is essential to optimal connectivity between brain regions and the examination of grey and white matter (WM) structures is crucial for a better understanding of the neurobiologic underpinnings of abnormal connectivity. Recently, neuroimaging studies have focused on WM structural alterations and investigators have applied different methods including region of interest approaches and diffusion tensor imaging (DTI).

Volumetric studies have focused on discrete brain regions and have identified abnormalities in several WM structures. Herbert et al. applied a novel MRI parcellation method and showed that WM volume was increased in the outer zone regions (closer to the cortical surface) with no significant differences in the inner zone regions (peri-callosal) (Herbert et al., 2004). In contrast, a recent study, using a different parcellation method, found no volumetric alterations in subjacent cortical white matter but reported reduced central white matter volume (peri-callosal) in children and adolescents with ASD (Jou et al., 2010). Reduction in central WM volume is consistent with several studies examining corpus callosum (CC) size (Hardan et al., 2000; Piven et al., 1997); and a recent meta-analysis substantiating this observation (Frazier and Hardan, 2009). Interestingly, this decrease in CC size appears to be persistent over time with recent evidence showing abnormal growth trajectory (Frazier et al., 2012). This observation is consistent with a longitudinal MRI study in very young children with ASD and reporting alterations in developmental rates in several brain regions including total WM volume (Schumann et al., 2010). These global alterations are also concordant with volumetric abnormalities in specific white matter tracts involving the right arcuate fasciculus, and the left inferior fronto-occipital and uncinate fasciculi (Radua et al., 2011). These converging observations provide evidence of the pervasiveness of WM volumetric alterations in ASD and the examination of the underlying neurobiology is warranted.

Abbreviations: AC–PC, anterior commissure–posterior commissure; CSI, chemical shift imaging; CC, corpus callosum; Cr, creatine; DTI, diffusion tensor imaging; FA, fractional anisotropy; FSIQ, full-scale intelligence quotient; GABA, gamma amino-butyric acid; GPC, glycerophosphocholine; ^{1}H MRS, proton magnetic resonance spectroscopy; IFT, inverse Fourier transformation; mI, myo-inositol; NAA, N-acetylaspartate; PC, phosphocholine; PCr, phosphocreatine; PD, proton density; SES, socio-economic status; STEAM, stimulated echo acquisition mode; TBV, total brain volume; WM, white matter.

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Similar to morphometric studies, investigations applying DTI techniques have consistently observed structural alterations in WM structures. Research studies utilizing DTI to examine microstructural properties of WM in children and adults with ASD have found alterations in fractional anisotropy (FA; coherent fiber tract directionality), as well as in mean and radial diffusivities. According to a recent review of 48 DTI studies in ASD, individuals with ASD tended to have decreased FA and increased radial diffusivity in many WM tracts of the brain, but most consistently in the CC, cingulum, and aspects of the temporal lobe (Travers et al., 2012). An exception to the reduced FA was found in a recent investigation of very young children (ages 1.8–3.3 years) who revealed increased FA in several brain regions including the arcuate fasciculus and CC (Ben Bashat et al., 2007) suggesting a possible developmental effect. More recently, a longitudinal study of children at high risk of developing ASD revealed increased FA in several white matter tracts in infants later on diagnosed with ASD compared to the control group before 12 months of age, but decreased FA in the ASD group at 24 months (Wolff et al., 2012). Findings from these DTI investigations indicate a pervasive pattern of WM disruption at the microstructural level and a better understanding of the neurobiologic underpinnings is needed.

The goal of this pilot investigation is to apply *in vivo* proton magnetic resonance spectroscopy (^{1}H MRS) to further examine white matter abnormalities in children and adolescents with ASD in comparison to age- and gender-matched neurotypical controls. *In vivo* ^{1}H MRS of the brain measures levels of N-acetylaspartate (NAA), phosphocreatine plus creatine (PCr + Cr) and glycerophosphocholine plus phosphocholine (GPC + PC) as well as glutamate and myo-inositol (mI) (Stanley et al., 2000). NAA is synthesized in neuronal mitochondria and metabolized in oligodendrocytes providing evidence of inter-compartmental cycling between neurons and oligodendrocytes (Baslow, 2000; Nadler and Cooper, 1972; Urenjak et al., 1993). Measurements of the PCr + Cr provide information on cellular energy metabolism with evidence indicating that Cr, after phosphorylation, is utilized as an energy reservoir in cells with high-energy demands (Sartorius et al., 2008). PC is a precursor and GPC a breakdown product of cellular membrane phospholipids. Collectively, GPC + PC reflects membrane synthesis and turnover associated with cellular entities such as the branching of dendrites and the formation of synapses (Dowdall et al., 1972; Stanley et al., 1995; Stanley et al., 2000). Finally, mI is believed to be an essential requirement for cell growth, an osmolite, and a storage form for glucose (Govindaraju et al., 2000) and has been proposed as a glial marker (Brand et al., 1993). We hypothesize that NAA will be lower in ASD, compared to controls. Therefore, application of ^{1}H MRS in measuring NAA and other metabolites may be of help in elucidating the neurobiologic underpinnings of white matter abnormalities in ASD.

2. Methods

2.1. Participants

Subjects were 17 boys with ASD and 17 healthy male controls between 8 and 15 years of age. Methodology of the study was approved by the Institutional Review Board. Written informed consent and assent were obtained from the parents and participants, respectively. All subjects had a full-scale IQ (FSIQ) of greater than 70. Participants with ASD represented all consecutive referrals to a research clinic who were eligible to participate in the pilot study and able to complete the imaging procedures. The diagnosis of ASD was established through the administration of the Autism Diagnostic Interview—Revised (ADI-R) and Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 2002; Lord et al., 1994) in addition to expert clinical evaluation. Children with autistic symptoms secondary to a specific etiology such as tuberous sclerosis or fragile X syndrome were excluded, as were potential subjects with evidence of genetic, metabolic, seizure, or infectious disorders.

Neurotypical controls were children recruited from the community through advertisements in areas socio-economically comparable to those of the families of origin of subjects with ASD. Healthy subjects were screened by face-to-face evaluations, questionnaires, telephone interviews, and observation during psychometric tests (please refer to the next section). Individuals with a family history (first degree relatives) of any neuropsychiatric disorder, such as ASD, 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 FSIQ > 70 and no learning disability as assessed by the Wide Range Achievement Test-R. Exclusions for control subjects and individuals with ASD were based on history and physical examination as well as laboratory testing when indicated.

Cognitive and behavioral phenotyping of participants included the Wechsler Intelligence Scale for Children-III (WISC-III), Social Responsiveness Scale (SRS), and Vineland Adaptive Behavior Scale (VABS). The SRS is a 65-item parent report questionnaire designed to measure the severity of ASD symptoms as they occur in natural social settings (Constantino et al., 2003). High scores in SRS indicate more severe behavioral symptoms. The VABS is a 433-item parent report questionnaire designed to assess personal and social skills needed for everyday living (Sparrow et al., 2005). Low scores in VABS suggest lower adaptive abilities. The WISC-III is an individually administered intelligence test for children between the ages of 6 and 16 that can be completed without reading or writing (Wechsler, 1991). The socioeconomic status (SES) of the family of origin was assessed using the Hollingshead method (Hollingshead, 1975).

Table 1 presents sample demographics and clinical characteristics. Participants with ASD and healthy controls did not differ on age or SES. However, there were significant differences in FSIQ. To more accurately estimate differences attributable to ASD diagnosis, age, SES, and FSIQ were included as covariates in mixed effects regression models. As expected, individuals with ASD showed significantly higher scores on the SRS and significantly lower scores across adaptive function domains. Scores on the diagnostic measures (ADI-R and ADOS) indicate that participants with ASD tended to have relatively high symptom levels. Finally, with the exception of one individual, all participants with ASD were taking psychotropic medications including selective serotonin reuptake inhibitors (N = 9), stimulants (N = 2), atomoxetine (N = 2), and atypical antipsychotics (N = 2). Five were taking other medications such as donepezil and buspirone.

Table 1
Sample demographics and clinical characteristics.

	ASD	Controls	t	p
	Mean (SD)	Mean (SD)		
N	17	17		
Age	12.5 (1.9)	11.6 (1.2)	1.68	.103
SES	4.5 (0.6)	4.4 (0.5)	0.60	.551
FSIQ	93.5 (18.3)	119.8 (11.3)	5.06	<.001
SRS total (T-score*)	84.1 (11.1)	41.1 (3.0)	14.04	<.001
Vineland (SS[a])				
Communication	94.2 (15.7)	104.9 (10.6)	2.31	.028
ADLs	80 (23.0)	102.6 (9.5)	3.74	.001
Social	76.2 (22.4)	107.3 (9.5)	5.25	<.001
Composite	81.3 (21.3)	106.5 (10.1)	4.37	<.001
ADI-R total	50.7 (8.3)			
ADOS total	15.3 (3.2)			

Abbreviations: ADLs, activities of daily living; ASD: Autism spectrum disorder; FSIQ, full scale intelligence quotient; SD, standard deviation; SES, socioeconomic status; Vineland, Vineland adaptive behavior scale.

* T-score = Mean of 50 and a standard deviation of 10.

[a] SS = Standard Score with mean of 100 and a standard deviation of 15.