MR Spectroscopy Suggests Hyperexcitability, Neuronal Injury, Inflammation and a Physiological Imbalance of Bioenergetics in White Matter of Children with Autism Spectrum Disorders

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Target Audience: Neurologists (Child Neurologists), Neuroradiologists, MR Physicists, MR Spectroscopists, Child Psychiatry and Neurodevelomental Specialists

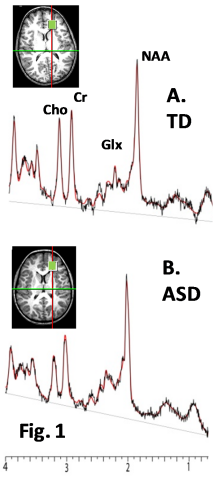
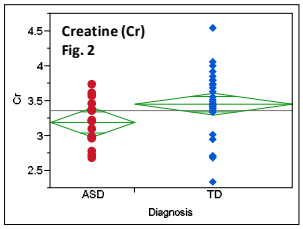


Fig. 1

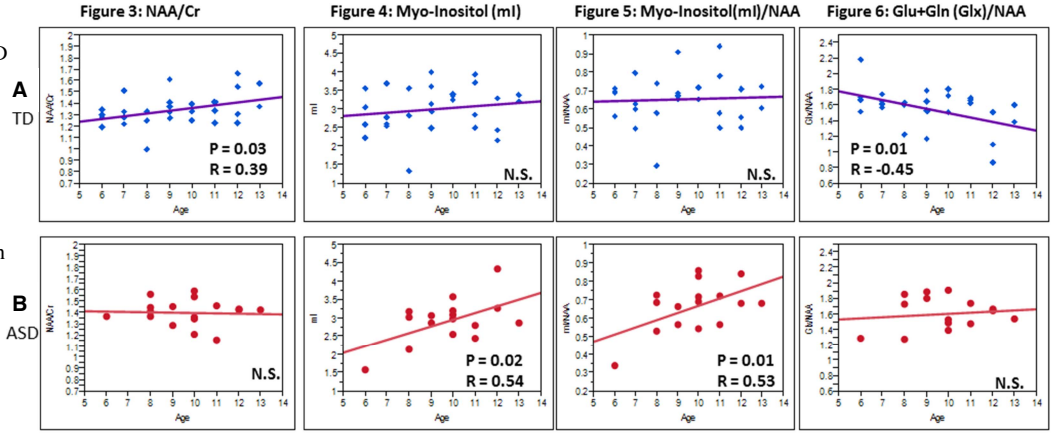
Purpose: Autism spectrum disorders (ASD) are a behaviorally defined syndrome considered to reflect complex interactions of genetic and environmental influences that alter normal brain development and function with enlarged white matter in childhood. A growing body of research in ASD suggests the presence of active pathophysiological disturbances such as increased excitation/inhibition (E/I) ratio, immune abnormalities, mitochondrial dysfunction and other bioenergetic disturbances.[1,2], These combined disturbances may be pertinent to the substantial risk of seizures in ASD which have a bimodal pattern of onset, one in the first two years of life and a second as entering puberty. Epilepsy prevalence rates are estimated between 15-38%,[3] with a higher prevalence of subclinical seizures.[4] MR Spectroscopy (MRS) provides a non-invasive method for characterizing biochemical and cellular metabolic states *in vivo*. Most ASD studies utilizing brain MRS report either no change or a decrease of N-Acetylaspartate (NAA) in both gray matter (GM) and white matter (WM), suggesting neuronal injury or mitochondrial dysfunction.[5] There are conflicting findings on choline containing compounds (Cho) and myo-inostol (mI) levels in ASD. Some but not all showed increases in Cho and mI, markers of gliosis and inflammation.[6] The same ambiguity was found for total creatine (Cr), a marker for energy metabolism, in ASD.[7] Glutamate (Glu) + glutamine (Gln), markers of neuronal excitation, are detected as an overlapping multiplet, (Glx), and have been found to be often decreased in ASD children often increased in ASD adults.[5] Increased Glu release or impaired clearance from interstitial space might contribute to an ongoing state of increased cerebral excitability/excitotoxicity and increased CNS irritability, giving rise to increased seizure risk.[4] ***Based on these prior findings, the objective of this study was to investigate biomarkers related to neuronal function, excitotoxicity, inflammation and perturbations of bioenergetics in the prefrontal WM in children with ASD and their age-matched typical development (TD) controls where volumetric differences have been most pronounced.***[8]

Methods: 53 male children ages 6 -13 yrs, participated. Data of sufficient quality for analysis were obtained from 17 (9.6 y ± 1.8) of 22 diagnosed with ASD and 30 of 31 TD subjects (9.3 y ± 2.2). Clinical diagnosis and phenotyping measures included an ADOS (Autism Diagnostic Observation Schedule) and measures of intelligence (verbal and non-verbal IQ). All MRI experiments were performed on a 3-Tesla MR imager (Siemens AG, Erlangen) using a 12-channel receive coil. Single voxel ^{1}H MR spectra were acquired from the left prefrontal WM region (VOI = 2 x 2 x 2 cm^3) using a point resolved spectroscopy sequence with TE/TR = 30/2500 ms. In addition, water unsuppressed spectra were acquired from the same region to estimate 'absolute' concentrations. Metabolite concentrations of NAA, Cho, mI, Cr and Glx were quantified using LCModel software. Figure 1A and 1B show typical voxel placements and spectra of a TD (9 y/o) and ASD (10 y/o) subject, respectively. Statistical analysis was performed in JMP 9. Two-way Student's t-tests were used to compare metabolite concentrations between ASD and TD subjects. Linear regression analysis was performed to estimate correlations between MRS metabolites and age in ASD vs. TD, controlling for age and/or non-verbal IQ.



Results:

- A comparison between ASD and TD subjects found lower Cr levels in the ASD subjects at all ages and independent of age or IQ (p=0.05, Fig. 2)
- Additionally, when we controlled for age and non-verbal IQ, we found significantly decreased NAA levels in ASD subjects vs. TD (p=0.048, not shown).
- TD children showed increases with age in NAA/Cr (p=0.03) Fig. 3A) but these remained unchanged in ASD children with age (Fig. 3B)
- mI significantly increased with age in ASD (p=0.01, Fig. 4B) but not in TD (Fig. 4A)
- Based on our mI and NAA findings we calculated changes in the mI/NAA ratio and demonstrating an increase of mI/NAA in children diagnosed with ASD (p=0.01, Fig. 5B), while no changes in mI/NAA could be detected in the TD cohort (Fig. 5A).
- Glx levels tended toward decrease in TD subjects with age (p=0.13) but remained unchanged in ASD subjects with age (NS; data not shown). We therefore investigated changes in Glx/NAA, showing a decrease in Glx/NAA in TD children with age (Fig. 6A), but no age-related changes in Glx/NAA in ASD (Fig. 6B).



Discussion: Our findings are consistent with the presence of active tissue pathophysiology in ASD, raising the related questions of how they arise and how they contribute to neurofunctional features of ASD. Cr differences may relate to altered energy metabolism. The lower NAA and Cr levels suggest neural injury and/or a state of energy deficit, potentially due to mitochondrial insufficiency in the face of the increased energy demands. Such higher demands could derive from the increased E/I ratio and excitotoxicity consistent with findings in our ASD group of persistently elevated Glx/NAA in ASD as well as from the inflammation suggested by increases in mI. The impact of the failure of Glx/NAA to decrease with age in ASD upon E/I ratio and excitotoxicity may also contribute to increased seizure onset in puberty in ASD. All of these metabolic changes alter the tissue substrate of the systems that generate information processing and behavior; how these impacts occur needs exploration. The role of such changes in white matter needs further exploration particularly since white matter changes are being found in other neuropsychiatric conditions such as psychosis and schizophrenia.[9]

Conclusion: MR Spectroscopy is a promising tool to study metabolic changes in ASD marking states of neuronal excitation, inflammation and bioenergetics. The utility of MRS in the future can be enriched by utilizing other MRS techniques such as 31Phosphorus spectroscopy and by incorporating MRS into multimodal imaging studies to address tissue and functional correlates.

References. 1. Rossignol & Frye Mol Psychiatry (2012) 17, 389. 2. Fatemi et al. Cerebellum, (2012). 3. Tuchman & Rapin Lancet Neurol (2002) 1, 352. 4. Spence & Schneider Pediatr Res (2009) 65, 599. 5. Dager et al. Mag. Reson Imaging (2008) 19, 81. 6. Chugani, Pediatric clinics of North America (2012) 59, 63. 7. Ipser et al. Metabolic brain disease (2012) 27, 275. 8. Herbert et al. Ann Neurol (2004) 55, 530. 9. Monin et al. CINP Conference Jerusalem (2013).