Global and regional brain metabolic deficits in autism

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Introduction Autism is a behaviorally defined developmental disorder characterized by language deficits, impairment in social and emotional reciprocity, and repetitive, restrictive interests and behaviour. Studies from various fields indicate that the disorder has a neurological origin, but the specific mechanism and developmental trajectory remain unknown. Characterization of neurobiological abnormalities among patients with autism is an important preliminary step in forming hypotheses regarding etiology, and to this end, numerous recent studies have examined the brains of autistic patients, though relatively few findings have been consistently replicated. The few postmortem studies in autism to date have found evidence for localized abnormal histology, particularly in the limbic system, cerebellum, brain stem nuclei, and cerebral gray and white matter (1,2). While imaging studies of brain anatomy in autism have also revealed regional abnormalities, especially in limbic structures and cerebellum, these findings have been inconsistent. A few recent studies (3,4) have investigated potential metabolic abnormalities in brains of children with autism using 1H-MRSI, though differences were subtle and distributed and no clear localized pattern of metabolite abnormality was evident. Estimating global gray and white matter metabolite levels rather than targeting specific localized regions of interest takes advantage of the full wealth of data available in MRSI examinations, and may allow for increased sensitivity in discerning subtle, distributed metabolic abnormalities in our sample of patients.

In the present study, children and adolescents with autism and a control group of healthy children were imaged using 1H-MRSI to evaluate global mean gray and white matter levels of proton MRS metabolites; metabolite levels in numerous targeted regions of interest were also quantified. We hypothesize that children with autism will differ in global gray and white matter N-Acetylaspartate (NAA) levels, reflecting widespread abnormal neuronal density.

Methods Twenty-seven boys with autism (ages 6-17 years) and 30 healthy boys (ages 6-17 years) were recruited from the local community. The groups did not differ in age, race, or handedness. Diagnosis of autism was made using the Autism Diagnostic Interview-Revised (ADI-R), the Autism Diagnostic Observation Schedule (ADOS-G), and by clinical observation. All patients met DSM-IV-TR criteria for autism as well as ADI-R and ADOS algorithm criteria. Patients were further assessed using the Wechsler Intelligence Scale for Children, 3rd Edition (WISC-III) or the Leiter International Performance Scale. Exclusionary criteria for patients included a non-verbal IQ below 70 or a history of seizures or any other neurological condition. Control subjects were recruited from the local community and were assessed with the Schedule for Affective Disorders and Schizophrenia-Childhood Version (K-SADS) to ensure that none had a major psychiatric disorder. Controls were also assessed with the WISC-III, and a full-scale IQ of less than 70 was exclusionary. No control participant had a personal history of neurological disorders. This study was approved by the local Health Sciences Research Ethics Board, and the parents or legal guardians of all participants provided written consent for participation in this study, while the participants provided written assent.

Participants were imaged with a 3T head-only MRI scanner (IMRIS, Winnipeg, Canada) equipped with a quadrature head coil. Details of the acquisition and analysis protocol are given in DeVito et al (5), and briefly outlined below. A 3D T1-weighted MP-RAGE image set, B1-map, and a two-slice 1H-MRSI image set (TI/TE/TR=230/135/1800 ms, 8x8x9mm nominal voxel size, 30 minute scan time, with circular k-space matrix) were acquired for each subject. The MRSI acquisition used CHESS and inversion recovery to suppress unwanted signals from water and extracranial lipid, respectively. MRSI data were quantified in the time-domain using prior knowledge from in vitro solutions of NAA, creatine (Cr), choline (Cho), glutamate + glutamine (Glx), and myo-inositol (Myo). Fitted metabolite amplitudes were corrected for coil load, radio-frequency field inhomogeneity, and cerebrospinal fluid partial volume effects. Estimates of 'global' metabolite levels in gray and white matter were made by first quantifying all spectra in each slice of each participant (~500 spectra per subject), as described above; data from the two slices were pooled. Fitted metabolite amplitudes for each voxel were then regressed against the fraction of gray matter in that voxel, determined by segmenting the co-registered high-resolution 3D image set. This resulted in estimates of mean gray and white matter metabolite levels for each participant. Localized MRS metabolite levels were also assessed from targeted single-voxel regions of interest, shown in Figure 1. Group differences in regional and global metabolite levels were investigated separately, using a linear mixed-model repeated measures analysis in each case. Age was used as covariate.

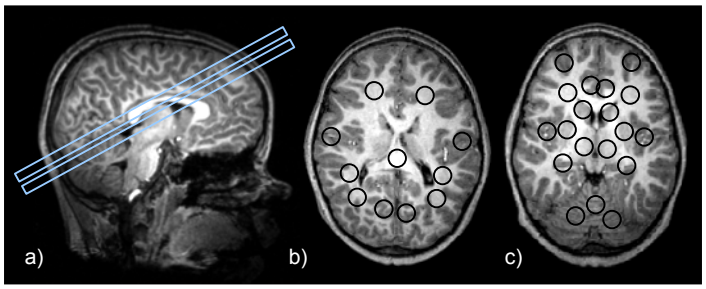

***Figure 1**: MP-RAGE images showing (a) MRSI slice positions and (b and c) targeted voxels selected for the region of interest analysis.*

Results Statistical analysis of averaged global metabolite levels revealed a significant group by tissue by metabolite interaction ($F_{13,292.4}$=52.2, p<0.0001), with this difference remaining significant after covarying for age ($F_{19,232.3}$=29.5, p<0.0001). Post-hoc ANCOVA of group differences in averaged global metabolite concentrations revealed reduced gray matter NAA (8%), Glx (10%), and Myo (8%) among patients with autism. There were no significant group differences in the concentration of any white matter metabolite, before or after covarying for age.

The regional analysis revealed significant group by region interactions for NAA ($F_{31,709.5}$=2.1, p=0.0007), Glx ($F_{31,754.9}$=1.5, p=0.048), and Myo ($F_{31,733.1}$=1.8, p=0.007). No significant main effects for group or higher-level interactions were seen for Cre or Cho. Post-hoc ANCOVA of group differences in NAA revealed reduced NAA among patients with autism in the left frontal cortex, left insular cortex, bilateral occipital gray matter, and left anterior white matter. Patients with autism also had reductions in Glx in the left frontal cortex, the right occipital cortex, and bilaterally in the thalamus. A significant reduction in Myo was seen in the right occipital cortex and left caudate nucleus.

Discussion Our patient sample exhibited distributed localized deficits in NAA and Glx in numerous cortical regions, with isolated deficits in frontal white matter and thalamus. Estimates of global tissue-specific metabolite levels revealed a significant pattern of reduced gray matter NAA and Glx. Given the putative role of NAA as a marker for functional neuronal density, these results suggest a global deficit in functional cortical neurons in our patient sample. Reduced gray matter Glx may reflect widespread abnormalities in glutamatergic circuitry. These observations are of particular interest in light of genetic studies reporting altered expression of brain glutamate receptors and transporters in patients with autism (6-8). Given the role of glutamate as the most abundant excitatory neurotransmitter, and the observed reduced cortical NAA, the findings of this study are in keeping with numerous functional studies reporting reduced cerebral metabolism and blood flow in patients with autism (9-12). While metabolite estimates from tissue classified as gray matter arise predominantly from cortical tissue, voxels from cerebellum and sub-cortical gray matter also contribute to these estimates. Further parcellation of the data set into sub-regions, such as brain lobe and hemisphere, may help further localize the observed metabolic deficits, while providing greater sensitivity than single-voxel region of interest analyses.

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Acknowledgements:
Hospital for Sick Children Foundation, NSERC
Child Health Research Institute, IMRIS, ORDCF