Brain Myo-Inositol in Children with Down Syndrome – an In Vivo Proton MRS study

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Introduction
Down Syndrome (DS) or trisomy 21, is a genetic disorder characterized by a multitude of morphological and functional abnormalities. One hallmark is mental retardation, becoming apparent during the first few months of life. The biochemical and physiological bases for the brain dysfunction are not well known. aberrantly over expressed proteins due to the extra chromosome 21 might provide clues to the etiology. Human Na⁺/myo-Inositol (mI) cotransporter gene (SLC5A3) was cloned and mapped to chromosome 21, whose product is an active mI transporter. Trisomic 21 cells may have an increased Na⁺/mI flux, if they are unable to curtail the transcriptional output from all 3 gene copies. The gene is under osmoregulatory control. Increased extracellular osmolality may lead to elevated mI in brain tissue because of the increased activity of SLC5A3. MI is a precursor of membrane phosphoinositides, playing an important role in e.g. calcium signaling and mitosis. The impact of a 50% increase in gene dosage is difficult to predict. Elevated ratio of mI to creatine in brain of adults with DS have been reported.

The purpose of this study was to determine whether the level of mI is increased in the brains of children with DS. We performed in vivo brain 'H-MRS and measured osmolality in a cohort of children with trisomy 21.

Materials and Methods
14 patients with trisomy 21, 6 males and 8 females, age range 1.2 to 13.6 years, and 13 control subject, 7 males and 6 females from 1.3 to 11.7 years old, underwent MRS studies of the brain. Patients with seizures, idiopathic developmental delay but otherwise without specific diagnoses and with normal brain MRI/MRS were selected into the control group. In 8 out of 14 DS patients plasma osmolality was determined. MRS studies were conducted on a 1.5T Siemens Magnetom Vision scanner. All spectra were obtained from the basal ganglia region. STEAM sequence was used with TR/TM/TE = 1600/15/10 ms, voxel size 2x2x2 cm³, NEX = 256. Water reference scans were obtained from the same ROI for eddy current correction. Metabolite concentrations in µmoles/g tissue were determined using a least squares estimation routine. We corrected for tissue density (1.05 g/cm³) and T1 effect, assuming a similar value for basal ganglia and gray matter (values of Kreis et al.).

Results
Results are summarized in Table 1. The mI concentration in basal ganglia in patients with DS is significantly increased compared to the control group. The Figure shows a spectrum of the basal ganglia in DS with the elevated mI peak compared to the spectrum of an age matched control subject. The osmolality (mean ± SD) in DS patients was 292 ± 6 mOsM.

Discussion
With in vivo 'H-MRS, we demonstrated a 28% increase of mI levels in basal ganglia in children with DS compared to an age matched control group. The plasma osmolality of all DS patient was within normal range. We hypothesize, that the increase of mI could be related to the presence of the extra copy of the SLC5A3 gene in the trisomic 21 cells. Studies on trisomy 16 mice, the animal model for DS reported elevated mI levels in brain, which support the hypothesis. The extra gene copy should result in a 50% increase in brain mI in DS patients, as has been demonstrated for mI levels in CSF in a group of adults with DS. An explanation for the discrepancy is not yet available. The 28% increase in brain mI could be pathophysiologically significant, if the increased influx of mI and Na⁺ leads to a perturbation in cell volume and sodium content or secondarily affects the levels of other relevant ions. Further studies on brain mI metabolism in DS patients are warranted.

Table 1: Summary of Results

<table>
<thead>
<tr>
<th></th>
<th>mI</th>
<th>Choline</th>
<th>Creatine</th>
<th>NAA</th>
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<tr>
<td>DS Patients</td>
<td>3.8 ± 0.5</td>
<td>1.6 ± 0.4</td>
<td>9.2 ± 1.3</td>
<td>7.8 ± 1.1</td>
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<tr>
<td>Control Group</td>
<td>2.9 ± 1.0</td>
<td>1.9 ± 0.3</td>
<td>9.9 ± 1.9</td>
<td>8.2 ± 1.1</td>
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Statistical Significance

<table>
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<th>Test</th>
<th>p-Value</th>
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<tr>
<td>t-test</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>two tailed</td>
<td>p = 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SD in units of µmoles/g tissue
't-test: two sample assuming unequal variances; 'two tailed p value

References:
2. Berry GT et al., Genomics 25, 507-13, 1995
8. Shetty Hu et al., Biochem J 313, 31-33, 1996
9. Shetty Hu et al., J Clin Invest 95, 542-46, 1995