

Down syndrome with and without dementia: and Alzheimer's disease in the general population.

An in vivo proton Magnetic Resonance Spectroscopy study.

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Abstract

It is poorly understood why people with Down syndrome (DS) are at extremely high-risk of developing Alzheimer's disease (AD) compared to the general population. One explanation may be related to their extra copy of risk factors modulated by chromosome 21. *Myo*-inositol (mI), whose transporter gene is located on chromosome 21, has been associated with dementia in the non DS population; however, nobody has contrasted brain mI in DS with (DS+) and without (DS-) dementia to other non-DS groups. Our primary aim was to compare the hippocampal concentration of mI ([mI]) and other brain metabolites such as N-acetylaspartate (NAA; a proxy measure of neuronal density and mitochondrial function) in DS+, DS- , and age-matched healthy controls using proton Magnetic Resonance Spectroscopy (¹H-MRS). We compared hippocampal [mI] and other metabolites in 35 individuals with genetically-confirmed DS [DS+ (n=17,age=53±6) and DS- (n=18,age=47±8)] to age-matched healthy controls (n=13,age=51±10) adjusting for proportion of the MRS voxel occupied by cerebrospinal spinal fluid, and grey/white matter. DS+ had a significantly higher [mI] than both DS- and healthy controls. In contrast neither DS+ nor DS- differed significantly from controls in [NAA] (although NAA in DS+ was significantly lower than DS-). Our secondary aim of comparing brain metabolites in DS+ and DS- to Alzheimer's disease (AD; n=39;age=77±5) revealed that the DS+ group had significantly elevated [mI] compared to AD or DS-. [mI] may modify risk for dementia in this vulnerable population.

Keywords: ¹H-MRS, Down syndrome, dementia, *myo*-inositol, N-acetylaspartate

1. Introduction

Down syndrome (DS), due to trisomy of chromosome 21, is one of the most common causes of intellectual disability of known genetic etiology. DS adults also have a high prevalence of age-related cognitive decline (Maatta et al., 2006) and Alzheimer's disease (AD). AD-type neuropathology (i.e., neuritic plaques and neurofibrillary tangles) occurs in the brain of all DS adults over 40 years of age (Wisniewski et al., 1985); with prevalence rates for clinically diagnosed AD increasing from 10% in the fourth decade of life to upwards of 75% in the sixth decade (Coppus et al., 2006; Schupf et al., 1998). It has been suggested that the increased risk for AD in DS results from trisomy of the amyloid precursor protein (APP) gene associated with AD which is located on chromosome 21 (Hardy & Allsop, 1991); however, other genes on chromosome 21 may also be relevant to dementia. *Myo*-inositol (mI) is a compound that affects neuronal osmolarity, signal transduction and cell survival; it has also been associated with amyloid deposition (McLaurin et al., 1998; Nitz et al., 2008). The mI transporter gene is located on chromosome 21 (Berry et al., 1995). Despite this, we are not aware of any case-control studies measuring brain mI in DS adults with (DS+) and without (DS-) dementia as compared to healthy control groups.

Proton Magnetic Resonance Spectroscopy (^1H -MRS) can be used to non-invasively measure concentrations of a number of brain metabolites including mI, *N*-acetylaspartate (NAA), creatine and phosphocreatine (Cr+PCr) and choline containing compounds (Cho). It is generally accepted that NAA is a marker of neuronal density and/or mitochondrial function. Cr+PCr are involved in phosphate metabolism and reflect energy use and storage by neurons. Cho is a measure of membrane synthesis and

turnover (for a review see van der Graaf, 2010). By using ^1H -MRS, a better understanding of the role of brain metabolites including mI may be obtained as it relates to trisomy of the mI transporter gene in DS+ and DS- populations.

Despite the very high risk for dementia in DS, only a very small number of ^1H -MRS studies have investigated this population. They reported that individuals with DS- have significantly increased [mI] within parietal (Huang et al., 1999) and hippocampal (Beacher et al., 2005) regions (but no differences in brain [NAA] or [Cr+PCr]; Shonk & Ross, 1995) as compared to non-DS controls. Age-related differences in [mI] within DS- have also been reported; with older individuals having higher levels of mI than their younger DS- counterparts (Huang et al., 1999). Furthermore, the increased hippocampal [mI] seen in DS- is associated with reduced overall cognitive ability (Beacher et al., 2005). Thus, it may be that increased [mI] could explain a proportion of both the intellectual disability and the increased risk for dementia in DS adults (Beacher et al., 2005; Shonk & Ross, 1995). Few studies, however, have investigated this. There is a single case report of an individual with DS+ who exhibited a pattern of elevated [mI] and lowered [NAA] within occipital gray matter that investigators described as ‘strikingly similar’ to AD (Shonk & Ross, 1995); however, there are no case-control ^1H -MRS studies of DS+ and DS-. Thus, the neurobiology of dementia in individuals with DS is poorly understood.

Although case-control ^1H -MRS studies of DS+ and DS- do not exist, there is evidence to suggest MRI structural differences between these two DS subgroups within key regions of the brain associated with AD-related neuropathology. Thus, the hippocampus, one of the brain regions first, and most severely affected by such

neuropathology, shows significant differences in volume between DS+ and DS- (Aylward et al., 1999; Beacher et al., 2009; Pearlson et al., 1998). Previously we have shown that the volume of the hippocampus correctly categorized 92% of individuals with DS- and 80% of individuals with DS+ (Beacher et al., 2009). Additionally, hippocampal volume in DS has been significantly and positively correlated with memory performance (Krasuski et al., 2002). While DS+ structural MRI hippocampal volume findings are similar to structural volumetric results reported in AD (de Leon et al., 1995; Thind & Sabbagh, 2007 for review), little is known about how ¹H-MRS metabolite abnormalities in DS compare to those associated with AD in the general population for this important region.

We used ¹H-MRS to compare the concentration of mI, NAA, Cr+PCr and Cho within the hippocampi of DS individuals with and without dementia to age-matched healthy controls. Our main hypothesis was that individuals with DS+ have significantly reduced [NAA] and increased [mI] within hippocampal regions as compared to individuals with DS- or controls. A secondary aim was to carry out a post-hoc comparison of DS+ to demented non DS individuals in the general population. This is the first report comparing AD to demented and non-demented DS populations and we hypothesized that [mI] will show a hierarchical pattern - such that levels of this metabolite will be highest in DS+, lowest in AD and place individuals with DS- between these two dementing groups (i.e., DS+>DS->AD).

2. Materials and Methods

2.1 Participants

In our main study we included a total of 48 adults with successful ^1H -MRS: 35 individuals with DS [17 with DS+ and 18 with DS-] and 13 healthy control subjects (Table 1). Individuals with genetically confirmed DS were recruited from community centers, residential homes and specialty clinics in London, Birmingham, Plymouth and Newcastle upon Tyne, UK. DS status was assessed in all participants by karyotyping and cognitive status was measured using the CAMCOG, a composite index of episodic memory, orientation, language, attention, praxis and executive function previously validated for use in DS (Hon et al., 1999). Given previously reported age-related differences in [mI] within DS- (Huang et al., 1999), we recruited age-matched healthy controls (HC) from general practice lists and the local population and confirmed the absence of dementia using the CAMCOG.

In order to carry out our preliminary comparison of dementia in DS to dementia in the general population, we recruited 19 males and 20 females with mild to moderate AD ($n=39$; $\text{age}=76.8\pm 4.9$, $\text{MMSE}=22.7\pm 3.7$) from a larger, national longitudinal study based at the Institute of Psychiatry (London). Individuals from this study were diagnosed with dementia using the ICD-10 Research Diagnostic Criteria with non-AD dementias excluded in keeping with NINCDS-ADRDA criteria (McKhann et al., 1984).

Insert Table 1 about here

All participants underwent standard physical, neurological and psychiatric screening, including routine clinical blood tests (e.g. renal, liver and thyroid function). In addition, all participants underwent clinical MRI to exclude other brain disorders including stroke or vascular dementia. Exclusion criteria included the presence of detectable physical (e.g., epilepsy and head injury) or psychiatric disorders (e.g. psychosis) affecting brain metabolism.

No participant was taking psychotropic medication at the time of the study. However, seven DS+ (41%) and 25 AD (64%) participants were taking acetylcholinesterase (AChE) inhibitors. Previous animal work suggests that AChE inhibitors increase PCr brain metabolism (Nakayama et al., 1996) while treatment with cholinergic agonists in adults with AD decreases Cho levels (Satlin et al., 1997); however, no studies to date have investigated the impact on other brain metabolites.

The study was approved by local and national Ethics Committees. After complete description of the study to the participant and identified carer, written informed consent was obtained, where possible. Where not possible, the participant's assent was obtained with formal consent provided by an identified carer.

2.2 ^1H -MRS Protocol

Subjects were scanned using a 1.5 Tesla, GE NV/i Signa MR system at the Maudsley Hospital, London. A vacuum fixation device ensured that subjects were both comfortable and restrained from movement during the scanning process. The whole brain was imaged with a three-dimensional (3-D) inversion recovery prepared fast spoiled gradient-recalled acquisition in the steady state (SPGR) T1-weighted dataset. These T1-weighted images were obtained in the axial plane with 1.5-mm contiguous

sections, repetition time (TR) of 13.8 milliseconds, inversion time (TI) of 450 milliseconds, echo time (TE) of 2.8 milliseconds and flip angle of 20° with one data average and a 256×256×124 matrix. Image contrast for all datasets was chosen with the aid of optimizing software (Simmons et al., 1996). Acquisition time was 6 minutes, 27 seconds. It should be noted that we had a very high success rate in MR scanning in our DS+ and AD groups with less than 20% drop out/non-compliance across all participants recruited with dementia.

¹H-MRS voxels of interest (VOI; 6-mL) were defined in the left and right hippocampi as illustrated by Figure 1(a). The anterior and posterior extents of the hippocampal/amygdala complex were initially defined from localizer images and a section of the axial 3-D inversion recovery prepared SPGR volume was then reoriented into the coronal plane for visualization of the hippocampus. The anterior extent of the voxel was defined as the coronal slice where the amygdala disappeared, with the posterior extent 20 mm from this. The center of the voxel was denoted by the center of the white matter tract in the superior/inferior and right/left positions. A point-resolved spectroscopy (PRESS) pulse sequence (TE 35 msec, TR 1500 msec, 256 data averages, 2048 points) with automated local shimming and water suppression and excellent reproducibility (Simmons et al., 1998) was used to obtain spectra from each voxel with high signal to noise ratio and clearly resolved NAA, mI, Cr+PCr and Cho peaks.

Water suppression was carried out by a sequence of chemical shift selective (CHESS) radio frequency (RF) pulses to excite and associated spoiling gradients to dephase water before the localization sequence. The flip angle of the last CHESS RF pulse was automatically adjusted to minimize the residual water signal. A flip angle of

greater than 90° was used to allow for T1 relaxation between the last CHESS RF pulse and the beginning of the localization sequence.

Insert Figure 1 about here

2.3 ^1H -MRS Data Analysis

Differences in proportions of white and grey matter in the ^1H -MRS voxels may confound group differences in metabolite concentrations. Thus to ensure that differences in tissue composition of the MRS voxels did not account for metabolic differences between subject groups, we segmented the 3-dimensional inversion recovery prepared spoiled GRASS dataset using statistical parametric mapping software (SPM; <http://spm.ion.ucl.ac.uk>) to determine the percentage of grey matter, white matter and CSF within the MRS voxel. The position of the ^1H -MRS voxels relative to the 3D dataset was determined automatically using in-house software.

^1H -MRS spectra were processed using LC-model on a Sun SPARC-10 workstation (Sun Microsystems Inc., Mountain View, CA). LC-model uses a linear combination of model spectra of metabolite solutions in vitro to analyze the major resonances of in vivo spectra. In this case, a basis set of alanine, aspartate, creatine, gamma-aminobutyric acid (GABA), glutamine, glutamate, glycerophosphocholine, mI, lactate, NAA, N-acetyl-aspartylglutamate (NAAG), scyllo-inositol, and taurine, together with a baseline function were used for analysis. Each spectra was reviewed to ensure adequate signal to noise ratio and linewidth, as well as the absence of artifacts. In addition, analysis was automatically corrected for CSF contamination of the MRS voxel

by dividing by the tissue fraction of the MRS voxel determined using SPM. These corrected concentrations were then calibrated to absolute millimolar units with respect to a phantom containing human in vivo levels of NAA, mI, Cr+PCr and Cho amongst other metabolites, at known concentrations, which was scanned using a PRESS acquisition with the same TE and TR after each subject visit (GE MRS phantom, GE, Milwaukee, WI, USA). T1 and T2 corrections were applied for each metabolite using literature values (Christiansen et al., 1993). As expected, many of the metabolite peaks included in the LC-model did not reach statistical significance when fitted; however those for NAA, mI, Cr+PCr and Cho did reach significant for all spectra derived from the hippocampi and concentrations were therefore derived from these metabolites. An example LC-model output is given in Figure 1(b).

2.4 Statistical analysis

Between-group comparisons of potential covariates including age, VOI proportion of grey and white matter were made using univariate general linear models (GLM) with follow-up least squares difference (LSD) testing. As previously stated, our data processing techniques incorporated a correction for the CSF component of the MRS voxel. Chi-square testing ruled out a difference in male/female inclusion by group.

No group violated assumptions of normality for NAA, mI, Cr+PCr and Cho concentrations when tested using the Kolmogorov-Smirnov statistic (all p-values >0.05). Thus, between-group comparisons of ¹H-MRS metabolites were made using univariate GLM analyses. Follow-up pairwise comparisons among estimated marginal means (adjusting for the covariate) were conducted where appropriate. Significance for all main effects was set at $p \leq 0.01$ with Bonferroni correction for multiple comparisons during

follow-up pairwise comparisons set at $p \leq 0.008$. Secondary analyses between DS+, DS- and AD followed a similar statistical protocol.

3. Results

3.1 Demographic Data Analyses

Separate paired sample t-tests determined there were no significant within-group differences in metabolite measurements based on side (i.e., left or right hippocampus). Thus, right and left hemisphere hippocampal measurements for each metabolite, i.e., NAA, mI, Cr+PCr and Cho, were averaged for a single measure of hippocampal metabolic functioning for each concentration.

As expected, age was not significantly different between DS groups and HCs (Table 1). However, the VOI proportions of grey matter [$F(2,43)=4.7$, $p=0.01$] and CSF [$F(2,41)=12.0$, $p<0.001$] were significantly different (Table 1) while the VOI proportions of white matter approached but did not reach significance [$F(2,43)=2.9$, $p=0.06$]. Thus, a composite index of the grey and white matter proportions of the MRS voxel [VOI proportions of grey matter/(VOI proportions of grey matter + VOI proportions of white matter)] were added as a covariate in all analyses. In addition to the composite VOI as a covariate, age was also added as a covariate in secondary analyses between DS+, DS- and AD groups given the significant difference in age between these groups, $F(2,71)=181.1$, $p<0.001$ (Table 1).

3.2 Main Analysis of between-group differences in DS (Table 1; Figure 2).

3.2.1 *Myo-inositol (mI)*: There was a significant main effect of group [$F(2,39)=9.3$, $p<0.001$; $\eta^2=.324$]. The DS+ group had a significantly higher [mI] concentration than

all the other groups (all p -values ≤ 0.003). Also, no other follow-up comparisons were significant (Table 1; Figure 2). The DS+ group had an increase in [mI] of approximately 13% compared to DS- and 19% compared to HCs (adjusted for age and grey/white matter proportions of the MRS voxel).

3.2.2 *N-acetyl aspartate (NAA)*: There was a significant main effect of group [$F(2,40)=5.7$, $p=0.006$; $\eta^2=.224$]. Follow-up comparisons revealed that the DS+ group had a significant reduction in [NAA] compared to the DS- ($p=0.002$) but not the HC group ($p=0.08$). The DS- group did not differ from HCs ($p=0.16$). There was an 11% reduction in adjusted [NAA] levels for the DS+ group when compared to the DS- group and a 6% reduction when compared to HCs.

Insert Figure 2 about here

3.2.3 *Other ¹H-MRS metabolites* – Neither analysis for creatine and phosphocreatine (Cr+PCr) nor Choline (Cho) revealed a significant main effect of group. Furthermore, controlling for total Cr+PCr in the above analyses did not alter the pattern of results for [mI]; however, the main effect of group for [NAA] was no longer significant.

As previously stated, the analyses outlined in section 3.2 were adjusted for age and grey/white matter proportions of the MRS voxel; however, similar results were obtained without correction for the volume fraction.

3.3 Secondary analysis of dementia in DS to AD in the general population.

Given the case report of DS+ in the literature which described levels of mI and NAA as being highly similar to AD (Shonk & Ross, 1995X), we re-ran our analyses comparing DS+, DS- and AD. There was a significant main effect of group for [mI] after controlling for age and a composite index of grey and white matter proportions of the MRS voxel, $F(2,61)=11.3$, $p \leq 0.001$, $\eta^2=.272$. Follow-up comparisons revealed significant elevations in this brain metabolite for the DS+ group compared to all other groups; the DS- group had significant elevations in [mI] compared to AD (i.e., $DS+ > DS- > AD$). The main effect of group for [NAA] was also significant, $F(2,58)=6.8$, $p= 0.002$, $\eta^2=.191$, with both demented groups (DS+ and AD) having significantly less hippocampal [NAA] than the non-demented DS- group.

4. Discussion

There is a single prior case report which used ^1H -MRS to investigate dementia in DS (Shonk & Ross, 1995) however there are no case-control studies. We compared, for the first time, ^1H -MRS metabolite concentrations in DS+, DS- and non-DS populations who did not differ significantly in age. Our results suggest that the hippocampal concentration of mI is significantly elevated in DS+ when compared to all other groups; whereas [NAA] levels were less discriminatory. Thus, while DS+ showed lower levels of [NAA] compared to DS-, neither DS group differed from HCs. Taken together, these results suggest that [mI] is a more appropriate target variable in delineating DS with and without dementia than other measurable brain metabolites including [NAA] within the hippocampus.

The threshold for expressing clinical symptoms of dementia in DS may be altered by elevated [mI] both directly and indirectly as a result of trisomy of the mI transporter gene location on chromosome 21. mI significantly affects neuronal development, survival, osmolarity, and membrane metabolism. As a result, elevations in [mI] may directly affect neuronal dysfunction and death. Indirectly, mI may affect neuronal function via a cascade of secondary changes at different levels of the signal transduction process and gene expression in the central nervous system. In addition, mI is a precursor to key phospholipids involved in calcium concentrations in brain and so may indirectly effect Ca^{2+} homeostasis (Yao et al., 2000); a process already implicated in the neurotoxic cascade of DS (Schuchmann et al., 1998) as well as AD (Emilsson et al., 2006). While increased [mI] may independently increase the risk for dementia, we suggest that it is more likely [mI] combines with other risk factors in DS (e.g., trisomy of the APP gene and/or hippocampal atrophy) to act as a synergistic mechanism for dementia in this vulnerable population. In keeping with this suggestion, *in vitro* studies have shown that inositol attenuates the neurotoxicity of amyloid (McLaurin et al., 2000).

While we suggest that it is likely the elevations we find in [mI] for DS+ are genetically determined by trisomy of chromosome 21, there are other plausible explanations. For example, group differences in gliosis and/or hydration may be impacting our results. Gliosis may increase [mI] in DS (Shonk & Ross, 1995), but this is unlikely to be the sole explanation for our results given that previous reports found that a proxy measure of gliosis (i.e., brain concentrations of Cr+PCr) was not related to [mI] in DS (Beacher et al., 2005). Furthermore, Cr+PCr was not significantly different between groups in our sample and controlling for this brain metabolite during analyses of [mI] did

not alter our pattern of reported results. It is also unlikely that the pattern of results for [mI] (which is affected by osmotic balance) can be explained by differences in water intake or hydration because subjects with abnormal renal function or urea and electrolytes levels or raised hematocrit were excluded from the study.

Some (Huang et al., 1999) but not all (Beacher et al., 2005; Murata et al., 1993) prior studies suggest that individuals with DS display age-related elevations in [mI] and/or reduced [NAA]. However, it is unlikely that age-differences can fully explain our results. Firstly, there were no significant differences in age between the DS+, DS- and HC groups but there were significant differences in [NAA] between DS+ and DS- and significant differences in [mI] between DS+ and all other groups. Thus, the extreme elevation in [mI] in DS+ as compared to all other groups does not appear to be age dependent. While previous studies report increased hippocampal atrophy in DS+ compared to DS- (Aylward et al., 1999; Beacher et al., 2009; Pearlson et al., 1998) and this may be reflected in our findings of reduced VOI proportions of grey matter for our DS+ compared to our DS- group, this is unlikely to be the sole explanation for our results given we controlled for this difference in our analyses. Further, there is now increasing evidence from this and other studies (Beacher et al., 2005; Huang et al., 1999) that non demented DS individuals have elevated [mI]. Hence one possibility is that some DS individuals have a greater elevation in [mI] than others, and this creates an inherently greater vulnerability in them to develop dementia. Nevertheless given that ours is an observational study we cannot address whether, within demented DS individuals, the further elevation in [mI] preceded or followed the onset of dementia. Future longitudinal studies are needed to directly assess this possibility.

A secondary aim of this study was to investigate brain metabolites across dementia populations involving DS and AD in the general aging population. Our preliminary results suggest the DS+ and AD groups showed significant, and comparable, reductions in [NAA] when compared to the DS- group and HCs, however, significant elevations in [mI] were only seen in DS+. This suggests a pattern of relatively less [NAA] reductions combined with significant elevations in [mI] only within DS+. This is in contrast to some (but not all) studies that have reported AD in non-DS populations is associated with an increase in mI concentration as well as a reduction in [NAA]. Our results may differ from prior reports because others; 1) investigated age-appropriate healthy control and at-risk groups compared to AD [mI] in temporal-parietal (Chantal et al., 2004) and/or parietal (Ackl et al., 2005) regions (i.e. *not* within medial temporal or hippocampal regions); 2) employed a higher field strength (i.e., 3 Tesla MRS; Wang et al., 2009) and; 3) investigated the *relative* amount of mI to other metabolites (e.g., ratios of mi/NAA; Wang et al., 2009) whereas we investigated the absolute concentration of mI within the hippocampus of individuals with either DS+, DS- or AD. While we cannot exclude the possibility of a role for mI in AD in the general population, the fact that levels of hippocampal mI in our AD group fell below that of either of the DS groups we studied suggests that *hippocampal* [mI] may pose a stronger biological risk for dementia in DS than for AD in the general population (Metastasio et al., 2006; Modrego et al., 2005). Despite the inherent difficulties obtaining age-matched DS samples for comparison to AD (Coppus et al., 2006) which prevented us from age-matching our groups, future studies need to examine differences in brain metabolites between dementia in the general population and DS individuals.

Given that a significant elevation in [mI] occurred within DS+ across both analyses (with DS- equal to HCs and intermediary between DS+ and AD), this suggests that the elevation in DS+ is unlikely to be a non specific effect of having dementia or DS in isolation. Rather, in our study sample, altered hippocampal [mI] may contribute more to a combination of dementia and Down syndrome. Hippocampal mI may be useful as a novel therapeutic target for dementia in DS much as manipulating other stereoisomers of inositol such as scyllo-inositol has shown promise in mouse models of AD (McLaurin et al., 2006) regardless of dementia staging (Fenili et al., 2007). Given that DS remains difficult to manage and treat effectively (Prasher, 2004), it is important to consider the contribution of mI to the early manifestation of dementia in this high risk population.

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Disclosures

We report no competing interests for any author of this manuscript.

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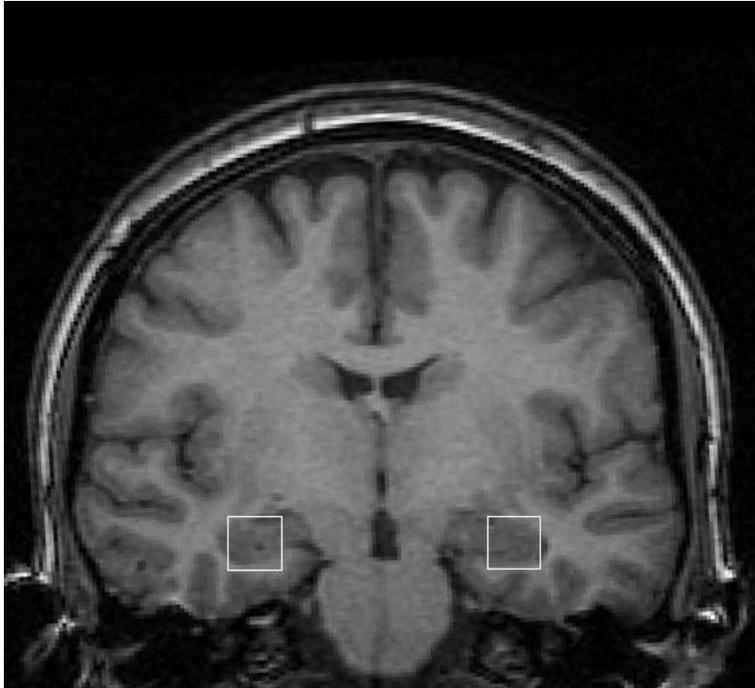
Table 1. Demographic, MRI and ¹H-MRS characteristics by group.

	DS+ (n=17)	DS- (n=18)	HC (n=13)	Summary of follow-up comparisons from univariate GLMs of DS+, DS- and HC analyses only	AD (n=39)
Age(years)	52.8±5.7	47.2±8.4	50.6±10.4	n/a	76.8±4.9
Sex (M:F)	10:7	14:4	10:3	n/a	19:20
CAMCOG**	29.4±16.8	51.6±19.3	119.0±2.6	HC > DS- > DS+	n/a
<u>MRI VOI</u> <u>proportions</u>					
White Matter	0.19±0.09	0.18±0.05	0.24±0.06	n/a	0.20±0.06
Grey Matter*	0.61±0.09	0.67±0.04	0.66±0.04	DS+ < DS- = HC	0.61±0.08
CSF**	0.19±0.08	0.13±0.04	0.08±0.03	DS+ > DS- > HC	0.18±0.09
<u>Metabolites</u> (absolute millimolar units)					
[NAA]**	7.1±0.2	7.9±0.2	7.5±0.2	DS+ < DS- only	6.6±0.6
[mI]**	6.6±0.2	5.8±0.3	5.4±0.3	DS+ > DS- = HC	4.9±1.0
[Cr+PCr]*	5.4±0.2	5.9±0.2	5.7±0.2	n/a	5.5±0.8
[Cho]	1.5±0.07	1.6±0.1	1.6±0.1	n/a	1.4±0.2

**p<0.001, *p<0.05; NOTE: Metabolite concentrations reflect estimated marginal means±standard error controlling for composite index of grey and white matter proportions of the MRS voxel; all other values reflect means±standard deviation with the exception of sex which is reported as raw totals for males:females. DS+=Down syndrome with dementia; DS-=Down syndrome without dementia; HC=healthy controls; AD=Alzheimer's disease; VOI=voxel of interest; [NAA]=N-acetyl aspartate; [mI]=*myo*-inositol; [Cr+PCr]=creatinine and phosphocreatine; [Cho]=choline; n/a=not applicable.

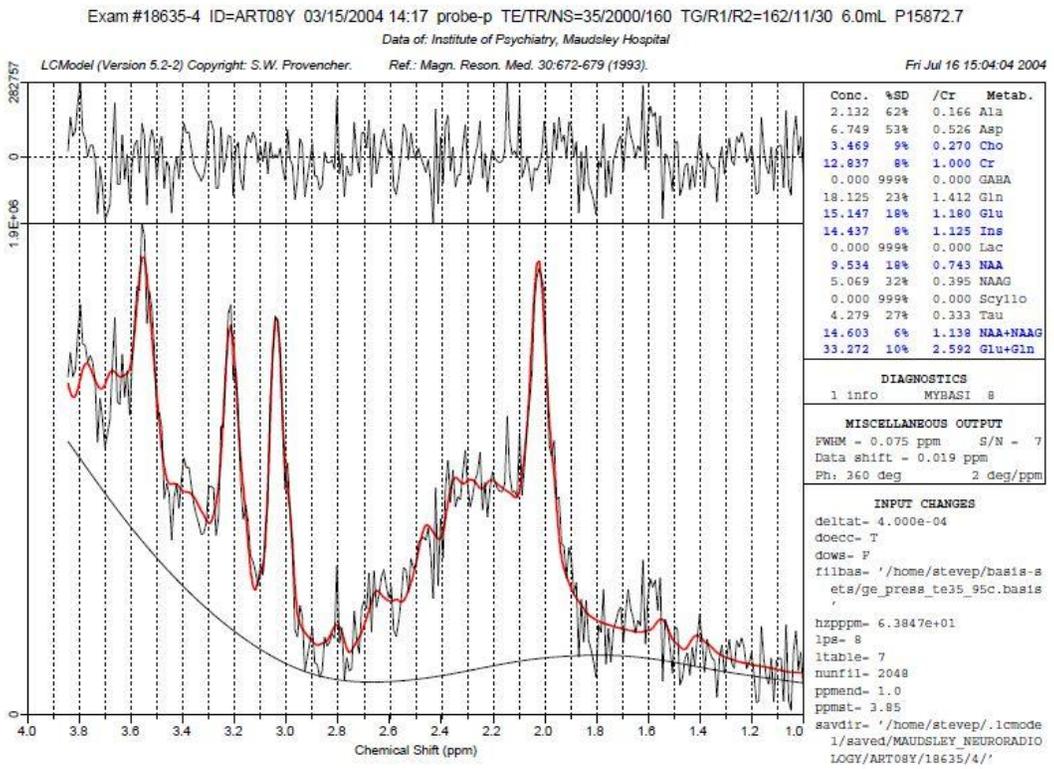
Figure 1. (a) T_1 weighted magnetic resonance image from a healthy subject illustrating the location of the ^1H -MRS voxels in the left and right hippocampi; (b) LC-model output for a typical spectrum. The main panel (bottom left) shows the fitted curve (red line) overlaid on the original data, with a smooth fitted baseline. The upper left panel shows the difference in signal between the fitted curve and the original data.

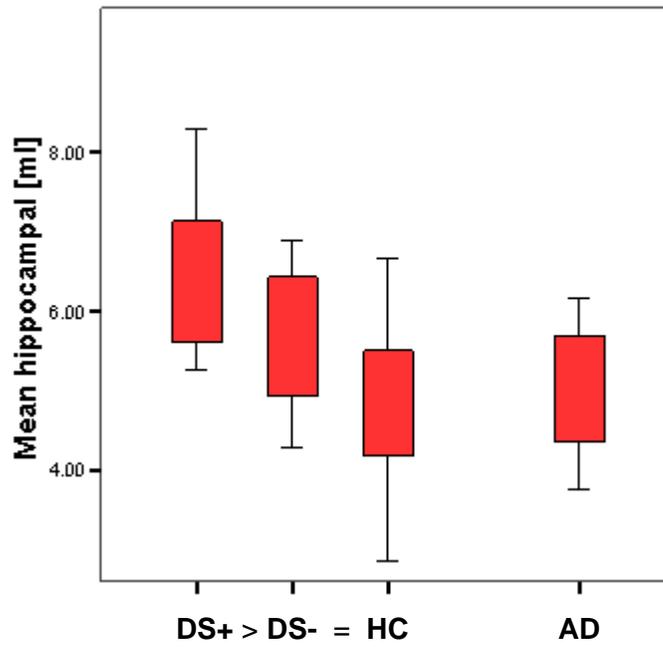
Figure 2. Boxplots of raw hippocampal concentrations of mI by group.



(1a)

(1b)





All p-values ≤ 0.003 ; see Table 1 for summary of follow-up comparisons between DS+, DS- and HC. NOTE: y-axis scale=absolute millimolar units; DS+=Down syndrome with dementia, n=17; DS-=Down syndrome without dementia, n=18; HC=age-matched healthy controls, n=13; AD=Alzheimer's disease, n=39.