Hippocampal *Myo*-inositol and Cognitive Ability in Adults With Down Syndrome

An In Vivo Proton Magnetic Resonance Spectroscopy Study

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Context: Down syndrome (DS) is the most common genetic cause of mental retardation. However, the biological determinants of this are poorly understood. The serum sodium/*myo*-inositol cotransporter gene is located on chromosome 21, and *myo*-inositol affects neuronal survival and function. Nevertheless, few in vivo studies have examined the role of *myo*-inositol in DS.

Objective: To determine if people with DS have significant differences in brain *myo*-inositol concentration from controls and if, within people with DS, this is related to cognitive ability.

Design: A case-control study.

Setting: Outpatient.

Participants: The sample was composed of 38 adults with DS without dementia (age range, 18-66 years) and 42 healthy controls (age range, 19-66 years). The DS and control groups did not differ significantly in age, sex, ethnic origin, apolipoprotein E status, or handedness.

Main Outcome Measures: Hippocampal *myo*-inositol concentration and cognitive performance, as measured by the Cambridge Cognitive Examination.

Results: Hippocampal *myo*-inositol concentration was significantly higher in people with DS than in controls ($P=.006$), and within people with DS, increased *myo*-inositol concentration was significantly negatively correlated with overall cognitive ability ($P=.04$).

Conclusions: Adults with DS have a significantly increased brain concentration of *myo*-inositol, and this is associated with reduced cognitive ability. Future studies are required to relate *myo*-inositol concentration in people with DS to brain development and increased risk for developing Alzheimer disease.

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DOWN SYNDROME (DS) IS associated with trisomy of chromosome 21 and is the most common genetic cause of mental retardation, occurring in approximately 1 in 1000 live births. The neuropathological features of Alzheimer disease (AD) occur in virtually all individuals with DS older than 40 years,[1] and the prevalence of dementia in people with DS in their 50s has been estimated at 66%.[2] This combination of preexisting mental retardation with a superimposed increase in dementia is difficult to treat and is an expensive management problem. However, the neurobiological basis of mental retardation and dementia in DS is poorly understood. One possible explanation may involve abnormalities in *myo*-inositol metabolism because the serum sodium (Na^+)/*myo*-inositol cotransporter gene (*SLC5A3*) is localized to chromosome 21,[3] and *myo*-inositol affects neuronal development and survival, cellular osmolarity, membrane metabolism, signal transduction, protein kinase C activation,[4] and amyloid deposition.[5] It has therefore been suggested that increased brain *myo*-inositol concentration may be related to cognitive impairment in DS.[6]

Proton magnetic resonance spectroscopy (^{1}H-MRS) can be used to measure in vivo brain concentrations of *myo*-inositol, *N*-acetylaspartate (NAA) (a marker of neuronal density[7] and/or mitochondrial function[8]), choline-containing compounds (Cho) (a measure of membrane synthesis/turnover[8]), and creatine and phosphocreatine (Cr + PCr) (a measure of cellular energy metabolism[8]).

There are few prior studies of people with DS using ^{1}H-MRS. Nonetheless, those that are available offer preliminary evidence that people with DS may have an increase in brain *myo*-inositol concentra-

Table. Demographic and Neuropsychological Characteristics and Mean Hippocampal Metabolite Concentrations of Adults With Down Syndrome Without Dementia and Healthy Controls*

	Adults With Down Syndrome (n = 38)	Controls (n = 42)	*P* Value
Demographics			
Age, y	35.2 (11)	34.6 (12)	.99
Male, No.	28	27	
Cognitive measures			
CAMCOG[15] total score	55 (22)	119 (3)	<.001
CAMCOG short-term memory score	11 (7)	22 (2)	<.001
Hippocampal ^{1}H-MRS			
Voxel gray matter %	0.45 (0.11)	0.44 (0.11)	.59
Voxel white matter %	0.49 (0.17)	0.53 (0.14)	.31
Voxel CSF %	0.05 (0.06)	0.04 (0.04)	.34
Mean *myo*-inositol concentration	5.35 (0.93)	4.78 (0.64)	.006†
Mean NAA concentration	7.66 (0.76)	7.68 (0.75)	.88
Mean Cho concentration	1.57 (0.23)	1.55 (0.22)	.66
Mean Cr + PCr concentration	5.68 (0.78)	5.75 (0.73)	.70

Abbreviations: CAMCOG, Cambridge Cognitive Examination; Cho, choline-containing compounds; Cr+PCr, creatine and phosphocreatine; CSF, cerebrospinal fluid; ^{1}H-MRS, proton magnetic resonance spectroscopy; NAA, *N*-acetylaspartate.

*Values are expressed as mean (SD) unless otherwise indicated.

†Statistically significant.

tion as compared with controls.[9-11] Previous ^{1}H-MRS studies of DS were important first steps; however, they used small samples, did not relate ^{1}H-MRS measures to cognitive performance, or did not measure metabolite concentrations in the hippocampus. Deficits in hippocampal function may be of particular importance to DS. For example, hippocampal volume is disproportionately reduced in the DS brain[12] and has been reported to be significantly positively correlated with memory ability in healthy adults with DS.[13] Also, the hippocampus is particularly affected by the neuropathological features of AD in adults with DS.[14] Therefore, we compared hippocampal neuronal integrity (as measured by ^{1}H-MRS) in adults with DS and healthy controls. Also, within people with DS, we investigated whether those metabolite concentrations that differed significantly between groups were related to overall cognitive performance, as measured by the Cambridge Cognitive Examination (CAMCOG).[15]

METHODS

PARTICIPANTS

We studied 38 adults with DS without dementia and 42 healthy controls. People with DS were recruited from cohorts in London, Birmingham, and Newcastle upon Tyne, England. Karyotyping was used to assess DS status in all participants. Dementia status was assessed using *International Statistical Classification of Diseases, 10th Revision* research criteria.[16] The DS and control groups did not differ significantly in age, sex, ethnic origin, apolipoprotein E status, or handedness (**Table**). The mean ages of the DS and healthy control groups were 35.2 years (range, 18-66 years) and 34.6 years (range, 19-66 years), respectively. Nineteen of the 38 participants with DS were older than 35 years at the time of scanning.

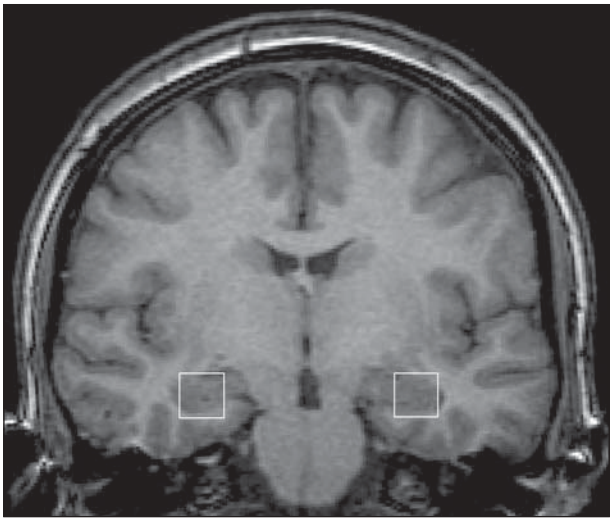

Figure 1. Axial T1-weighted magnetic resonance image from a healthy control subject, illustrating the locations of proton magnetic resonance spectroscopy voxels in the left and right hippocampi.

All participants underwent standard physical, neurological, and psychiatric screening, including routine blood tests (eg, renal and liver function tests, complete blood cell count, and thyroid function tests) and clinical magnetic resonance imaging. We excluded people with physical or psychiatric disorder affecting brain function (eg, hypertension), or a known history of birth trauma or head injury. In addition, we excluded subjects whose clinical magnetic resonance images suggested brain damage (for example, as indicated by the presence of white matter hyperintensities). None of the participants was taking psychotropic medication at the time of the study. The project was approved by the institutional review board, and after complete description of the study to the participants, written informed consent, or assent, was obtained from them or their caregivers.

DATA ACQUISITION

Participants and controls were scanned using a 1.5-T GE Signa MR system (General Electric, Milwaukee, Wis). A VacFix vacuum fixation cushion (S&S X-Ray Products Inc, Brooklyn, NY) was used to ensure that participants were both comfortable and restrained from movement during the scanning process. This device allows the cushion to be molded closely to the patient's head, comfortably minimizing subject movement.

Differences in proportions of white and gray matter and cerebrospinal fluid (CSF) in the ^{1}H-MRS volume of interest may confound group differences in metabolite concentrations. Thus, to ensure that differences in tissue composition did not account for metabolic differences between subject groups, we acquired an axial 3-dimensional inversion-recovery prepared fast spoiled gradient recalled acquisition in a steady state T1-weighted data set (repetition time, 13.8 milliseconds; inversion time, 450 milliseconds; echo time, 2.8 milliseconds; flip angle, 20°; 1 data average; image matrix, 256×256×124; and 1.5-mm sections). Acquisition time was 6.27 minutes.

In the left and right hippocampi, ^{1}H-MRS voxels of interest (6 mL) were defined (**Figure 1**) using a previously published location method.[17] A point-resolved spectroscopy pulse sequence (echo time, 35 milliseconds; repetition time, 1500 milliseconds; 256 data averages; 2048 points) with automated shimming and water suppression was used to obtain spectra from

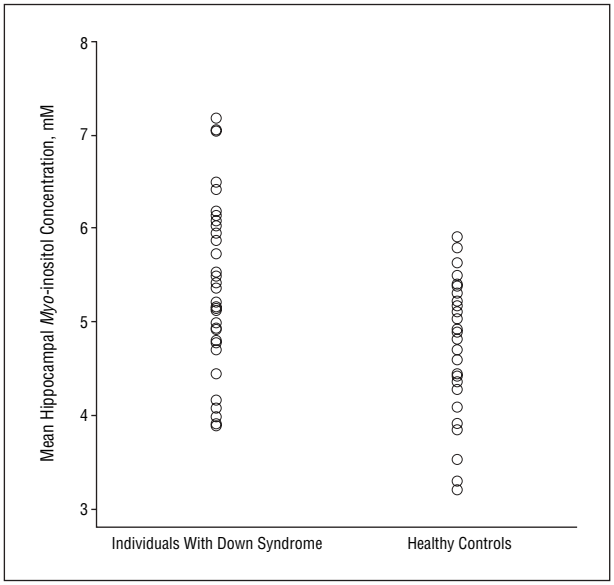

Figure 2. Mean hippocampal *myo*-inositol concentration in individuals with Down syndrome without dementia and healthy controls. One-way analysis of variance indicated that participants with Down syndrome had significantly higher concentrations of *myo*-inositol (*P*=.006).

each voxel with a high signal-noise ratio and clearly resolved *myo*-inositol, NAA, Cho, and Cr + PCr peaks.[18]

DATA ANALYSIS

The ^{1}H-MRS concentrations were derived using LC model on a Sun SPARC-10 workstation (Sun Microsystems Inc, Mountain View, Calif). LC model uses a linear combination of model spectra of metabolite solutions in vitro to analyze the major resonances of in vivo spectra.[19] A basis set of concentrations of alanine, aspartate, creatine, γ-aminobutyric acid, glutamine, glutamate, glycerophosphocholine, *myo*-inositol, lactate, NAA, *N*-acetyl-aspartylglutamate, *scyllo*-inositol, and taurine, together with a baseline function, were used for analysis. As expected, many of these metabolite peaks that were included in the LC model did not reach statistical significance when fitted. However, *myo*-inositol, NAA, Cr + PCr, and Cho concentrations did reach statistical significance for all spectra derived from the hippocampi, and concentrations were therefore derived from these metabolite peaks.

To ensure that differences in tissue composition did not account for metabolic differences between subject groups, the tissue composition of each ^{1}H-MRS voxel was analyzed using SPM (Statistical Parametric Mapping) software[20] to determine the percentage of gray and white matter and CSF composition.

COGNITIVE ASSESSMENT

Cognitive ability was measured using the CAMCOG.[15] The CAMCOG has been validated for use with adults with DS[21] and provides a measure of general cognitive function, including measures of episodic memory (which is associated with hippocampal function), orientation, language, attention, praxis, and executive function. The CAMCOG is appropriate for assessing cognitive function in people with mental retardation, unlike more standard tests of cognitive function such as the Wechsler Adult Intelligence Scales. The CAMCOG did not produce ceiling or floor effects, with the exception of a small number of CAMCOG subtests.

The British Picture Vocabulary Scale[22] was used to test receptive vocabulary (which is highly correlated with full-scale IQ) to give an additional indication of overall cognitive function.

For each participant, neuropsychological testing was completed within 6 months of scanning.

STATISTICAL ANALYSIS

Analysis of ^{1}H-MRS metabolites was carried out using SPSS (SPSS 8.0 for Windows; SPSS Inc, Chicago, Ill). Normality of distribution was assessed in both groups and tested for significance using the Kolmogorov-Smirnov statistic. Neither group violated the assumption of normality. The data were normally distributed; therefore, parametric tests of difference and correlation were used. Group differences in metabolite concentrations were tested with 1-way analysis of variance, with group as the between-subject factor. There were no significant interactions between the side from which ^{1}H-MRS metabolites were measured (left or right hippocampus) and group or between the effect of *myo*-inositol on cognitive function and side. Therefore, mean hippocampal metabolite values were considered in the analysis.

We examined the relevant scatterplots and there was no evidence of nonlinearity. Therefore, Pearson correlation coefficient (2-tailed) was used to assess linear correlations between metabolite concentrations and cognitive function and metabolite and age in both groups.

RESULTS

The DS group had a mean mental age of 6.7 years (range, 3.2-16.10 years) as measured by the British Picture Vocabulary Scale.

There were no significant group differences in the tissue composition of the ^{1}H-MRS voxels in mean white matter, gray matter, and CSF. Also, within the DS and control groups, there were no significant correlations between ^{1}H-MRS measures and mean white matter, gray matter, and CSF volume of the ^{1}H-MRS voxels.

Compared with healthy controls, individuals with DS had a significantly higher mean hippocampal concentration of *myo*-inositol (12.4%; $F_{1,76}$=8.154; *P*=.006) (**Figure 2**). There were no significant between-group differences in any other ^{1}H-MRS measure (Table). After covarying for voxel gray matter, white matter, and CSF, the significant between-group difference remained in the mean hippocampal *myo*-inositol concentration ($F_{1,63}$=5.910; *P*=.02).

Within the DS group, we carried out a planned exploration of the relationship between overall cognitive ability (as measured by total CAMCOG score) and brain *myo*-inositol concentration (**Figure 3**). There was a significant negative correlation between mean *myo*-inositol concentration and overall cognitive ability (*r*=–0.337; *P*=.04). We then carried out further exploratory post hoc analyses and related different domains of the CAMCOG to *myo*-inositol concentration. There was a significant negative correlation between mean *myo*-inositol concentration and total praxis score of the CAMCOG (*r*=–0.396; *P*=.01). The negative correlation between mean *myo*-inositol concentration and overall cognitive ability remained significant after the CAMCOG praxis score was excluded from the data set (*r*=–0.329; *P*=.046). There was also a trend toward a negative cor-

relation between mean *myo*-inositol concentration and total memory score of the CAMCOG ($r = -0.297$; $P = .07$). However, this additional correlation did not survive Bonferroni correction. Within the DS group, there were no significant correlations between CAMCOG total score and the voxel content of gray and white matter or CSF, and the correlation between *myo*-inositol concentration and CAMCOG total score remained significant after partialling out tissue segmentation measures.

There were no significant correlations between ^{1}H-MRS measures and age in either group (linear or nonlinear), and there were no significant group × age interactions for any ^{1}H-MRS measure. Within the DS group, the linear correlation (Pearson *r*) between mean *myo*-inositol concentration and age was 0.219 ($P = .18$), and within the healthy controls group, it was 0.172 ($P = .30$).

COMMENT

We found that adults with DS without dementia have a significantly higher hippocampal *myo*-inositol concentration than healthy controls, and within individuals with DS, *myo*-inositol concentration was significantly negatively correlated with overall cognitive performance. The significance of this correlation was not affected when potential outliers were dropped, and the result was not driven by differences in the normality of the data. We therefore suggest that a proportion of cognitive impairment in DS may be related to differences in brain *myo*-inositol concentration.

Brain tissue composition of ^{1}H-MRS voxels (in terms of gray and white matter) can affect metabolite measurements. However, we found no significant between-group differences in the proportion of gray matter, white matter, or CSF contained in the ^{1}H-MRS voxels or significant correlations between tissue segmentation measures and ^{1}H-MRS measures. In addition, the group difference in *myo*-inositol concentration remained significant after correcting for tissue segmentation measures. Thus, it is unlikely that differences in voxel composition can fully account for the significant between-group differences we found in *myo*-inositol concentration.

Our sample of individuals with DS did not have significant white matter hyperintensities, as assessed by clinical magnetic resonance imaging. This suggests that additional brain damage (for example, associated with postoperative hypoxic-ischemic brain injury) was not a significant confound in our DS sample. Further, it is unlikely that the group difference in *myo*-inositol concentration (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 hematocrit were excluded from the study.

Because of the demands of complying with the scanning procedure, our sample included few subjects with DS with a profound degree of mental retardation. Our results may therefore not be generalizable to this subgroup of individuals with DS.

The cause of the elevation in *myo*-inositol concentration in the DS brain, and how this affects cognitive ability, is unknown. It is possible that the (approximately 12% increase we found in the *myo*-inositol concentration does not directly affect hippocampal neuronal function but simply reflects another underlying metabolic process that is more closely linked with neuronal dysfunction. For example, it has been proposed that an elevated *myo*-inositol concentration in the DS brain reflects a gliotic process whereby neuronal degeneration is associated with an increase in the number of glial cells.[9] Astrocytes, the most common type of glial cell, have a relatively high concentration of creatine.[23] However, after we covaried for concentration of Cr + PCr, the elevation in *myo*-inositol concentration in the DS group remained highly significant. Thus, the increase in *myo*-inositol concentration in the DS brain is probably not fully explained by gliosis. Further, there was no significant between-group difference in hippocampal neuronal density or mitochondrial metabolism or membrane turnover (as measured, respectively, by NAA and Cho concentrations) and/or the proportion of the hippocampal voxel occupied by gray and white matter or CSF. Thus, these factors are unlikely to pose significant confounds for our analysis. However, a significantly increased uptake of *myo*-inositol has been reported in cultured fibroblasts of individuals with DS.[24] Thus, on the balance of probabilities, it is likely that the increase in brain *myo*-inositol concentration in people with DS is related to an up-regulation of *myo*-inositol transport associated with an extra copy of the Na^+/*myo*-inositol cotransporter gene in trisomy 21 neurons.

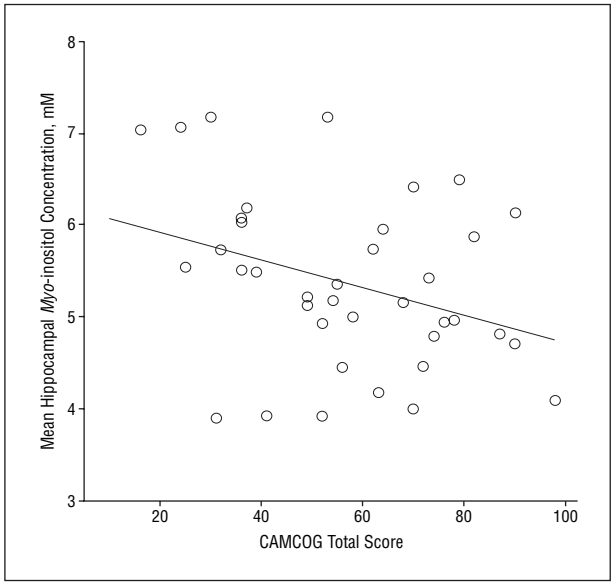

Figure 3. Mean hippocampal *myo*-inositol concentration and overall cognitive function, as measured by total Cambridge Cognitive Examination[15] (CAMCOG) score in individuals with Down syndrome without dementia.

One previous ^{1}H-MRS study of individuals with DS reported an approximately 50% increase in brain *myo*-inositol concentration.[11] The authors of that study proposed that *myo*-inositol concentration in the DS brain is subject to a gene-dose effect because there is also a 50% increase in the dose of the Na^+/*myo*-inositol cotransporter gene in DS cells. Our results do not support this suggestion because we found an approximately 12% increase in *myo*-inositol concentration. Similarly, 2 other previous ^{1}H-MRS studies of individuals with DS also re-

ported increases in the brain *myo*-inositol concentration that were substantially less than 50%.[9,10] Hence, increased brain *myo*-inositol concentration, associated with having a 50% increase in the transporter gene, is probably modulated by various downstream effects. For example, there may be other genes localized to chromosome 21 that also affect the phosphoinositide system (eg, synaptojanin1[25]) and whose overexpression in DS also affects brain *myo*-inositol concentration.

We found that within adults with DS without dementia hippocampal *myo*-inositol concentration was significantly negatively correlated with overall cognitive ability, as measured by the CAMCOG total score. However, this is an observational study and we can therefore only report an association, as opposed to causation. Hypotheses concerning the potential mechanism(s) underlying a possible causal relationship must be speculative. Nevertheless, abnormally high *myo*-inositol concentration is likely to disrupt a number of cell processes in the brain, including osmoregulation and membrane metabolism. *Myo*-inositol is also a key precursor in many neurotransmitter systems, in the phosphoinositide signal transduction system, and also in neuronal calcium signaling. Calcium signaling, in turn, modulates a large number of aspects of brain development and function such as neurotransmission, learning, and memory.[26] Abnormalities in calcium signaling are also associated with apoptosis, which may be increased in trisomy 21 neurons.[27] Thus, variations in brain *myo*-inositol concentration may directly affect neuronal development, survival, and function. Also, *myo*-inositol may initiate a cascade of secondary changes at different levels of the signal transduction process and gene expression in the central nervous system. Thus, there are a number of potential mechanisms whereby increased *myo*-inositol concentration in the DS brain may be causally linked to neuronal dysfunction.

We do not propose that differences in *myo*-inositol concentration explain all of the cognitive impairment in DS. There are a number of other important factors, such as the quality of educational environment and individual genetic makeup.[28] Further, we did not examine the neuronal integrity of other brain regions critical to higher cognitive function. This was owing to the time constraints involved in scanning people with mental retardation. Also, we only found a trend to a significant relationship between *myo*-inositol concentration and more specific measures of hippocampal function (eg, episodic memory scores). The results of previous ^{1}H-MRS studies of individuals with DS suggest that increased *myo*-inositol concentration also occurs in other brain regions. Hence, we suggest that increased brain *myo*-inositol concentration may explain a proportion of the cognitive deficits in people with DS but that this is most likely not a "hippocampal-specific" effect. Rather, it is likely that there is a generalized increase in brain *myo*-inositol concentration in the DS brain. This is difficult to assess in people with mental retardation owing to patient compliance and time constraints. Nevertheless, future studies are required using multivoxel magnetic resonance spectroscopy approaches.

We did not include participants with dementia or AD. Thus, we can only speculate on whether increased *myo*-inositol concentration may predispose to the later development of dementia. *Myo*-inositol is amyloidogenic,[5] and there are numerous reports of an association between AD in the general population and increased brain *myo*-inositol concentration, as measured by ^{1}H-MRS.[29] People with DS invariably develop europathological features of AD when over the age of 40 years, even in the absence of dementia, and may therefore be expected to have an age-related increase in brain *myo*-inositol concentration. Consistent with this expectation, Huang and colleagues[11] reported that brain *myo*-inositol concentration in adults with DS without dementia significantly increases with age. They suggested that this age-related increase in *myo*-inositol concentration in people with DS (superimposed on a trait-related increase) reflects a "predementia" phase in which the neuropathological features of AD are accumulating but which precedes loss of neurons. However, we are unable to support this suggestion because we did not find that *myo*-inositol concentration was related to age in either the DS or the healthy control groups, despite our DS sample being larger (38 compared with 19) and having a wider age range than that studied by Huang and colleagues (18-66 years compared with 28-62 years). Nevertheless, it remains possible that significantly higher hippocampal *myo*-inositol concentration in adulthood predisposes individuals with DS to later development of AD, even if this does not further increase in older age.

It has been hypothesized that in the DS brain the presence of an extra copy of the amyloid precursor protein gene (which is localized to chromosome 21) leads to abnormalities in amyloid precursor protein processing in neuronal membranes and subsequently to amyloid plaques and AD.[30] However, *myo*-inositol also promotes the formation of amyloid plaques.[5] Therefore, the predisposition of people with DS to amyloid deposition and subsequent AD may arise from a significantly higher gene dose of both the amyloid precursor protein and the *myo*-inositol transporter genes. Nevertheless, our sample did not include people with DS and AD, and there are no studies in the world literature examining between-group differences in *myo*-inositol concentration in people with DS with and without AD. Thus, we cannot determine if, or how, brain *myo*-inositol concentration relates to AD in individuals with DS.

We found no evidence that individuals with DS without dementia have differences from controls in neuronal density and/or mitochondrial function, as measured by NAA concentration. Also, we found no evidence that individuals with DS without dementia have differences from controls in neuronal membrane turnover, as measured by Cho concentration. However, phosphorus magnetic resonance spectroscopy would be a more suitable technique for assessing membrane metabolism than ^{1}H-MRS. Finally, we found no evidence that individuals with DS without dementia have differences from controls in cellular energy metabolism, as measured by Cr + PCr concentration. It is possible that people with DS have differences in neuronal integrity that we were unable to detect, but our results suggest that the largest detectable contribution to cognitive impairment, from the metabolites measured using ^{1}H-MRS, comes from increased *myo*-inositol concentration.

CONCLUSIONS

Adults with DS without dementia have a significant increase in brain *myo*-inositol concentration. Within individuals with DS, increased *myo*-inositol concentration is significantly associated with reduced overall cognitive ability. Also, increased *myo*-inositol concentration may predispose people with DS to the later development of AD, possibly mediated by promotion of β-amyloid plaques. There is, at present, no treatment for cognitive impairment in DS. However, the possibility that increased *myo*-inositol concentration in the DS brain may be associated with a greater degree of mental retardation and/or later AD suggests that trials are required to determine whether reduction in brain *myo*-inositol concentration improves cognitive outcome in DS.

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