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^{1}H-MRS Metabolites in Adults with Down Syndrome: Effects of Dementia

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^{1}H-MRS metabolites in adults with Down syndrome: Effects of dementia

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ABSTRACT

To determine if proton magnetic resonance spectroscopy (^{1}H-MRS) detect differences in dementia status in adults with Down syndrome (DS), we used ^{1}H-MRS to measure neuronal and glial metabolites in the posterior cingulate cortex in 22 adults with DS and in 15 age- and gender-matched healthy controls. We evaluated associations between ^{1}H-MRS results and cognition among DS participants. Neuronal biomarkers, including *N*-acetylaspartate (NAA) and glutamate-glutamine complex (Glx), were significantly lower in DS patients with Alzheimer's should probably be changed to Alzheimer (without ' or s) through ms as per the new naming standard disease (DSAD) when compared to non-demented DS (DS) and healthy controls (CTL). Neuronal biomarkers therefore appear to reflect dementia status in DS. In contrast, all DS participants had significantly higher *myo-inositol* (MI), a putative glial biomarker, compared to CTL. Our data indicate that there may be an overall higher glial inflammatory component in DS compared to CTL prior to and possibly independent of developing dementia. When computing the NAA to MI ratio, we found that presence or absence of dementia could be distinguished in DS. NAA, Glx, and NAA/MI in all DS participants were correlated with scores from the Brief Praxis Test and the Severe Impairment Battery. ^{1}H-MRS may be a useful diagnostic tool in future longitudinal studies to measure AD progression in persons with DS. In particular, NAA and the NAA/MI ratio is sensitive to the functional status of adults with DS, including prior to dementia.

1. Introduction

Down syndrome (DS) is a developmental disorder involving triplication of chromosome 21 and is one of the most common causes of intellectual disability of known genetic etiology. Memory processes are affected early in the course of aging in DS, and nearly all adults with DS show sufficient neuropathology for a diagnosis of Alzheimer' disease (AD) by their fifth decade of life (Mann and Esiri, 1989; Wisniewski et al., 1985). Interestingly, despite the presence of AD neuropathology, typically by age 40 years, dementia may not be observed until almost a decade later (Zigman, 2013). Neuronal loss (Sadowski et al., 1999), reduced neurotransmitters (Schliebs and Arendt, 2011), and increased neuroinflammation (Wilcock and Griffin, 2013; Wilcock et al., 2015a) may play important roles in the development of dementia and compromising cognition in DS. To identify biomarkers and critical pathological cascades associated with dementia and develop novel interventions to slow disease progression, it is critical to develop diagnostic strategies that enable early detection of the underlying neurobiological changes in DS.

Proton magnetic resonance spectroscopy (^{1}H-MRS) has been widely used to characterize neurochemical brain health and disease. In particular, the neuronal markers of *N*-acetylaspartate (NAA) and glutamate-glutamine complex (Glx), and glial marker of myo-inositol (MI), correspond to disease severity and often correlate well with clinical variables in aging and AD (Parnetti et al., 1997; Lin and Rothman, 2014). Specifically, neuronal loss or injury can be indicated by lower than normal levels of NAA and Glx, while neuroinflammation with activated astrocytes and microglia in brain disorders are associated with elevated MI (Chang et al., 2013). To further distinguish the neuronal-glial interplay the ratio of NAA to MI has been used for studies of sporadic AD and has been useful for distinguishing nondemented from demented people (example - Fig. 5 in (Lin et al., 2005)).

In DS, decreased NAA and increased MI have been observed in the hippocampus of adults by MRS (Beacher et al., 2005; Lamar et al., 2011) and in an early report of one individual with DS in the posterior

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parietal cortex (PCC) (Shonk et al., 1995). Interestingly, in a larger study using MRS in people with DS with and without dementia, hippocampal measures of Glx did not distinguish these two groups, and neither was different from controls (Tan et al., 2014).In this study, we hypothesized that signatures of neuronal health would be reduced, and those of inflammation increased in DS as a function of cognitive status particularly in the PCC as it is a region where hypometabolism is observed in adults with DS (Haier et al., 2003). Our long-term goal is to develop in vivo ^{1}H-MRS criteria for future clinical settings that enable early identification of neurochemical differences, prediction of dementia development, and consequently treatment efficacy, in adults with DS. We measured brain metabolites using ^{1}H-MRS and correlated them with cognitive scores in adults with DS who are enrolled in a longitudinal study of aging and dementia at the University of Kentucky.

2. Materials and methods

2.1. Participants

MRS measures were collected from the baseline visit of an ongoing longitudinal study of adult DS evaluating decline in cognitive functioning and neural integrity as predictors of the development of dementia (Powell et al., 2014). We recruited participants older than 35 years through local DS support groups and residential facilities in Kentucky and southern Ohio from 2010 to 2014. We excluded participants if they had active and unstable medical conditions (e.g., cardiovascular complications). Because thyroid dysfunction is common in individuals with DS, we included these participants if their thyroid dysfunction was medically controlled. The study cohort included 22 adults with DS (Table 1). We also recruited 15 age- and gender-matched (by frequency matching) non-DS control participants (CTL). CTL reported no history of significant neurologic, cardiovascular, or psychiatric disorders. All participants completed informed written consent or assent with guardian consent. The study and research procedures were approved by the University of Kentucky Institutional Review Board.

2.2. Neurocognitive and behavioral measures

Expert consensus review of each participant with DS determined dementia diagnosis. Briefly, two neurologists and two neuropsychologists applied NINCDS-ADRDA criteria for dementia (McKhann et al., 1984) and reviewed all data from medical history, medical and neurologic examinations, laboratory tests, structural imaging, mental status measures, and informant report of any changes in functional status and activities of daily living. The purpose of the consensus conference is to reach a single diagnosis through review of each participant's information. Therefore, clinical ratings reflect a group decision among the 5 clinicians. As for informant-based rating scales, each individual's primary care provider completes the behavioral assessments (e.g., affect, Activities of Daily Living (ADL)). The care provider, identified as the participant's guardian or person with daily contact if in a group or institutionalized setting (at least 8 h per week) is interviewed and remains the primary informant at each scheduled study visit.

Dementia duration is based on the primary caregiver's report of the age at onset of cognitive and ADL changes (subtracted from the age at which the scan is obtained). Hence, persons recruited into the study with dementia (verified in clinical consensus) have a longer duration than participants who develop dementia after study enrollment (shorter duration and greater precision of onset age). In addition, some participants enroll in the study given caregiver concerns about cognitive and ADL change. This results in a shorter duration value.

We obtained Dementia Questionnaire for Persons with Mental Retardation (DMR) ratings from informants for each participant with DS in addition to the objective mental status measures for diagnostic confirmation (Evenhuis, 1996). The DMR was developed in the 1990s by Evenhuis and colleagues (Evenhuis, 1992) as a standardized screening

Table 1
Demographics of study participants.

Participant characteristics	All DS	DS	DSAD	CTL	DS vs DSAD	DS vs CTL
N	22	17	5	15		
Age (mean, SD)	48.3 (7.6)	46.7 (7.9)	53.7 (3.2)	47.8 (7.6)	n.s.	n.s.
Gender (men/women)	6/16	6/11	0/5	6/9		
BPT (mean, SD)	70.2 (8.8)	72.7 (7.5)	59.5 (4.7)*	N/A	$p < 0.01$	N/A
SIB (mean, SD)	80.3 (18.0)	85.9 (13.8)	61.2 (18.5)	N/A	$p < 0.05$	N/A
DMR (mean, SD)	16.3 (18.2)	9.3 (7.5)	40 (24.5)	N/A	$p < 0.001$	N/A
Dementia Duration (y; mean, SD)**		N/A	2.54 (2.69)	N/A		

Key: BPT, Brief Praxis Test; CTL, non-DS controls, DMR, Dementia Questionnaire for Persons with Mental Retardation; DS, Down syndrome; DSAD, Down syndrome with dementia; SD, standard deviation; SIB, Severe Impairment Battery.
N/A Not applicable.
* One person could not complete the BPT.
** Dementia duration is estimated based on onset of symptoms derived from clinical interviews.

tool for dementia using caregiver report. It consists of eight subscales that are combined into a total score and also yields subscores for cognition and social functioning. Each item is rated on the degree of deficit (0 = none, 1 = moderate, 3 = severe) such that increasing scores reflect a greater degree of disability (0–104 for the total DMR).

Further, we derived premorbid levels of functioning from individual case files of existing academic and psychological test records, medical records, as well as family member interviews. Based on this information participants were categorized as low, medium, and high functioning based upon their level of intellectual disability (Lott, 2011). Premorbid level of functioning in the current sample included 13 with mild ID and 9 with moderate ID. All participants with DS completed medical and cognitive assessments.

The Brief Praxis Test (BPT) (Dalton and Fedor, 1997) and the Severe Impairment Battery (SIB) (Panisset et al., 1994) were used as neuropsychological outcome measures for the present study. Both measures have demonstrated usefulness in tracking progressive decline due to dementia in DS (Lott et al., 2012). The SIB is a mental status scale that was specifically developed to track AD progression during more advanced stages of dementia. The current SIB version consists of 51 items across nine domains (social interaction, memory, orientation, language, attention, praxis, visuospatial, constructional abilities, and orientation to name). The scale has a maximum score of 100, has been used in clinical trials in DSAD (Lott, 1822; Prasher et al., 2002), and has shown good test-retest and criterion validity when used in DS (Witts and Elders, 1998). The BPT is derived from the Dyspraxia Scale for Adults with Down Syndrome (Dalton et al., 1999) and consists of 20 items that evaluate both gross and fine motor functions. This 80-point scale has demonstrated good reliability and sensitivity to change in DS and DSAD (Sano et al., 2005).

2.3. ^{1}H-MRS data acquisition and analysis

^{1}H-MRS measurements were obtained immediately following acquisition of neurocognitive measures. Participants were scanned on a 3T TIM Siemens scanner at the Magnetic Resonance Imaging and Spectroscopy Center at the University of Kentucky. The single ^{1}H-MRS voxel of interest (VOI; 80 cm^3) was defined a priori in the posterior cingulate cortex as confirmed by MPRage to be consistent with reports by Ross and colleagues (Lin et al., 2005)(PCC; Fig. 1). High-resolution, 3D anatomic images were acquired using an MP-RAGE sequence [repetition time (TR) = 1690 ms, echo time (TE) = 2.56 ms, flip angle (FA) = 12°, 1 mm isotropic voxels, 6:19 min]. The rationale for selecting this brain region was two-fold (Mann and Esiri, 1989): to reduce the impact of movement artifacts, which can be a concern with imaging people with DS and (Wisniewski et al., 1985); to select a brain region that is sensitive to mild cognitive impairment and AD in the general population in previous MRS studies (Lin et al., 2005; Tumati et al., 2013). A Stimulated Echo Acquisition Mode (STEAM) sequence was used with repetition time (TR) of 1500 ms and echo time (TE) of 35 ms, flip angle = 90°, 128 averages and 1024 points; automated local shimming and water suppression (Simmons et al., 1998). The rationale for using STEAM was to allow us to compare our current results with ongoing studies of MRS in people without DS with mild cognitive impairment and AD at our imaging center and previous publications (Tumati et al., 2013; Murata et al., 1993).

^{1}H-MRS spectra were processed and the concentrations of the metabolites were derived using LCModel on a Linux operating system. LCModel uses a linear combination of model spectra of metabolite solutions in vitro to analyze the major resonances of in vivo spectra (Provencher, 1993). For each spectra, a signal to noise ratio was calculated by LCModel and a cut off of greater than 6 was used (range was 6–17) and the full width half maximum estimate of linewidth averaged 55.7 ppm. Data points for which the LCMODEL provided a % standard deviation (for the fit) of lower than 15% for Cre, MI, NAA/NAAG (range was 3–15%) and lower than 20% for Glx peaks (range 10–20% except for one DS participant at 30%) were included in the analysis. The automatic advanced DESS sequence was used to shim the spectroscopy voxel. Shimming and gradient QA is conducted on the scanner bimonthly to ensure reproducibility. The metabolites that consistently reached our signal to noise ratio and % standard deviation included Cr, MI, Glx (combined Glu and Gln) and NAA with NAAG (the NAA resonance at 2 ppm contains both NAA and *N*-acetylaspartylglutamate (NAAG). We report results here reflecting the combination of NAA and NAAG, though we use the term of NAA for brevity. The concentration of all the ^{1}H-MRS metabolites was normalized to that of Cr as described in previous reports (summarized in (Tumati et al., 2013)).

2.4. Statistics

Statistical analyses were performed using GraphPad Prism (GraphPad, San Diego, CA, USA) and IBM SPSS Statistics (Version 22). Mean differences in metabolites, volunteer demographics and cognitive test scores among the three groups (DS, DSAD, CTL) were evaluated using one-way analysis of variance (ANOVA) with Tukey's Multiple Comparisons (and nonparametric ANOVA; Kruskal-Wallis). In addition, Pearson correlation coefficients were used to explore associations between metabolites and mental status. Values of $p < 0.05$ were considered significant.

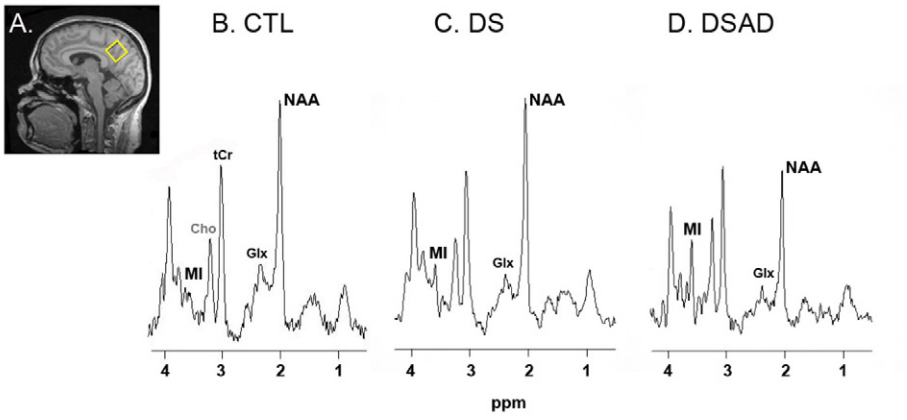

Fig. 1. ^{1}H magnetic resonance spectroscopy study design and spectra. A. The inset image illustrates the PCC region VOI used for MRS measures, on an MPRAGE image. The graphs are examples of spectra of B. control (CTL), C. nondemented Down syndrome (DS) and D. Down syndrome with Alzheimer's dementia (DSAD) participants. Cho: choline; Cr: creatine; Glx: glutamate-glutamine complex; MI: myo-inositol; NAA: N-acetylaspartate; ppm: parts per million.

3. Results

3.1. Demographic characteristics and neurocognitive measure outcomes

Table 1 displays the demographics and group means on the BPT and SIB for the DS groups. As expected, there were no significant age differences across the groups. Among the 22 adults with DS, 5 females (but no males) were identified with dementia due to Alzheimer's disease (DSAD). To address possible confounding due to having only females in the DSAD group, we compared metabolite levels in the control group between males and females and observed no significant differences (data not shown). Similar results were obtained when comparing males and females in the DS group. However, samples sizes in this study preclude strong conclusions regarding gender differences as has been reported in MRS studies in sporadic AD. DSAD participants had significantly lower scores on the BPT ($t = -3.32$, $p = 0.0036$) and SIB ($t = -3.26$, $p = 0.0039$) compared to DS participants without dementia (referred to as DS). Levels of intellectual disability prior to a diagnosis of dementia did not differ between the participants with DS and DSAD (Fisher's exact test $p = 0.45$) as the sample reflected a 50%/50% split of participants in the mild and moderate ranges overall and a 20%/30% split for those persons diagnosed with dementia.

3.2. Differences in neuronal and glial biomarkers in the DS participants

Fig. 1 shows the representative spectra from each group. The DSAD participants had reduced NAA and Glx but elevated MI compared to the other two groups. NAA, at 2.0 ppm, is an amino acid derivative synthesized in neurons and transported down axons. Therefore, it is an almost 100%-specific marker of viable neurons, axons, and dendrites (Lin et al., 2005). Glx, which lies between 2.1 and 2.4 ppm, is a mixture of glutamate and glutamine, which is closely involved in excitatory/inhibitory neurotransmission and the mitochondrial redox system. As a result, Glx provides a marker in MRS for neural integrity. MI, which resonates at 3.5 ppm, may represent glial activation (as an osmoylte that maintains glial cell volumes) (Chang et al., 2013) as well as membrane metabolism (Lamar et al., 2011). It is therefore used as a putative glial biomarker.

The quantitative results shown in Fig. 2 further revealed the differences between DS, DSAD, and CTL groups. The neuronal biomarkers, Glx (Fig. 2A; $F_{2,34} = 4.225$, $p = 0.02$) and NAA (Fig. 2B; $F_{2,34} = 19.98$, $p < 0.0001$) were significantly lower in DSAD participants, but no differences were found between DS and CTL ($p > 0.05$). However, in the glial biomarker, we found that MI in both DS and DSAD patients was significantly higher relative to CTL (Fig. 2C; $F_{2,34} = 22.64$; $p < 0.0001$), but no difference was found between DS and DSAD ($p > 0.05$).

3.3. Neuronal-glial metabolism shifts in DS

The NAA/MI ratio distinguished CTL, DS, and DSAD groups by ANOVA ($F_{2,34} = 29.33$; CTL vs. DS: $p < 0.001$; CTL vs. DSAD: $p < 0.001$; DS vs. DSAD: $p < 0.01$; note: Kruskal-Wallis Test, statistic = 21.404; $p < 0.0001$). CTL had the highest value (2.4 ± 0.4), followed by DS (1.7 ± 0.3 ppm), and DSAD had the lowest value (1.1 ± 0.1 ppm) (Fig. 2D). However, using a stepwise linear regression, using the NAA value alone provides the best predictor for distinguishing demented vs nondemented people with DS compared with the NAA/MI ratio ($r^2 = 0.608$). In contrast, a similar regression analysis including all DS and

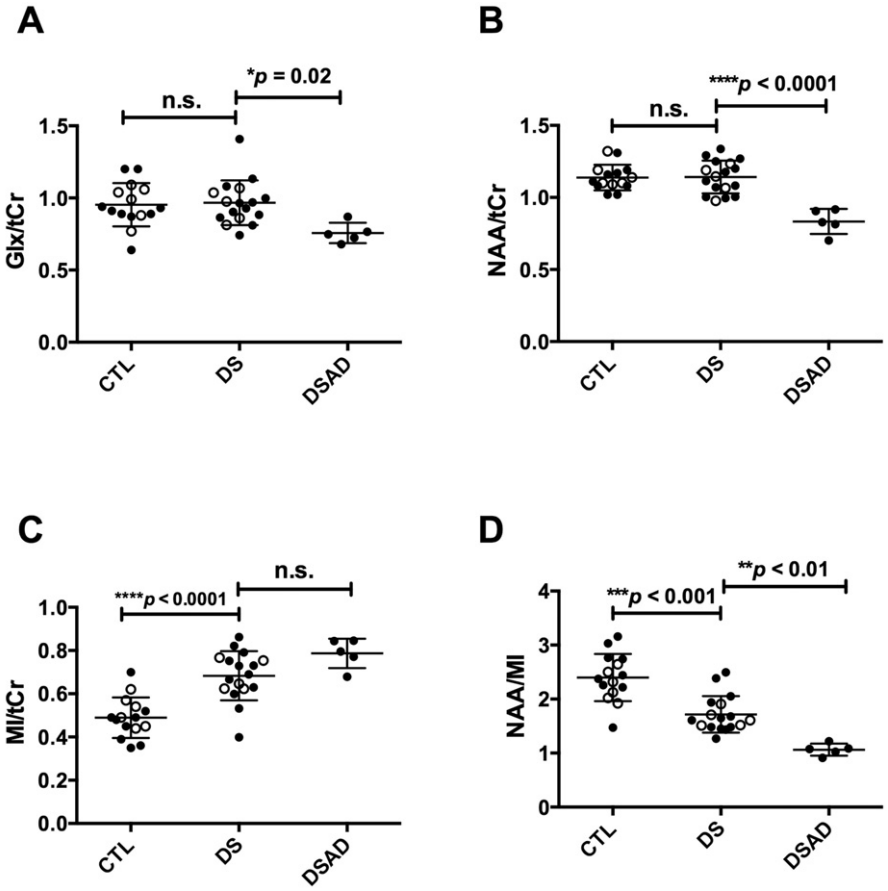

Fig. 2. Brain metabolite differences in DS and DSAD participants. (A) and (B) DSAD participants had significantly lower Glx and NAA compared to the other two groups; (C) Down syndrome participants, whether demented or not, had significantly higher MI relative to the CTL; (D) CTL, DS and DSAD showed significantly different NAA/MI ratio. All the metabolites were normalized to creatine. Glx: glutamate-glutamine complex; MI: myo-inositol; NAA: *N*-acetylaspartate. n.s. = non-significant. Bars represent SEM. Females are shown as closed circles and males as open circles.

control participants, the relationship changes and the ratio of NAA/MI is a better predictor of dementia than NAA alone ($r^2 = 0.633$).

Fig. 3 shows the relationship between NAA and MI among the three groups. A combination of low NAA and high MI clearly separated DSAD individuals from the other two groups, suggesting that NAA values lower than 1.0 and MI values higher than 0.65 (i.e., NAA/MI < 1.54) may be a key threshold for discriminating DS with AD from DS without AD. Further, DS without dementia had overlapping NAA values with CTL, but most were over 0.55 values for MI.

3.4. Brain metabolites and cognition associations

To test the hypothesis that reduced neuronal and increased glial metabolites by MRS would be associated with poorer cognition in DS, we used Pearson correlations unadjusted for multiple comparisons. One person with DS who was demented could not complete the BPT task. Fig. 4 shows individual test scores for participants in the study and highlights those with (open circles) and without dementia (closed circles). We found that higher NAA values were associated with higher BPT (Fig. 4A; $r = 0.65$, $p = 0.002$) and SIB (Fig. 4B; $r = 0.60$, $p = 0.003$) scores; BPT was also positively correlated with Glx (Fig. 4C; $r = 0.45$, $p = 0.040$) and NAA/MI (Fig. 4D; $r = 0.50$, $p = 0.022$). SIB scores were positively correlated with NAA but not with Glx or the NAA/MI ratio. Given that the presence/absence of dementia is confounded with cognitive test scores, we also calculated correlations when only the nondemented participants were included in the analysis. The correlation between BPT and NAA remained significant ($r = 0.485$ $p = 0.048$) and the SIB correlation with NAA was marginally significant ($r = 0.456$ $p = 0.066$). The correlations between BPT and Glx or NAA/MI were not significant suggesting that people with dementia are primarily responsible for driving the association between BPT and Glx or NAA/MI.

4. Discussion

In the current study, we show data that suggest that ^{1}H-MRS of the posterior cingulate cortex could be a powerful tool to differentiate between aging and dementia in DS. We found that DS participants (including those with and without dementia) had an overall higher MRS marker of glial inflammation (MI) compared to the CTL group. These results indicate that people with DS, whether demented or not, had a shifted neuron-glial metabolism (reduced NAA and increased MI); the shift was more pronounced in the demented DS individuals than the non-demented DS adults. Further, MI overlapped to some extent in the DS and DSAD groups suggesting that either MI increases are already present over the age of 35 years in DS and may be developmental or an early aging event. Increased MI in younger adults with DS in the hippocampus has been reported suggesting this is a phenotype of DS (Beacher et al., 2005). The NAA/MI ratio could be an index to predict the risk for dementia in DS adults especially given that there was no overlap between the two groups in their NAA/MI ratios. Thus people with DS, whether demented or not, may have neuroinflammatory processes active after 35 years of age compared to non-DS healthy controls, and neuronal function loss may be the key factor associated with dementia in DS participants. In addition, the DSAD participants also had a lower MRS marker of neuronal integrity (NAA) than the DS and CTL participants. The NAA/MI ratio further differentiated CTL, DS, and DSAD, with reduced levels in the demented group.

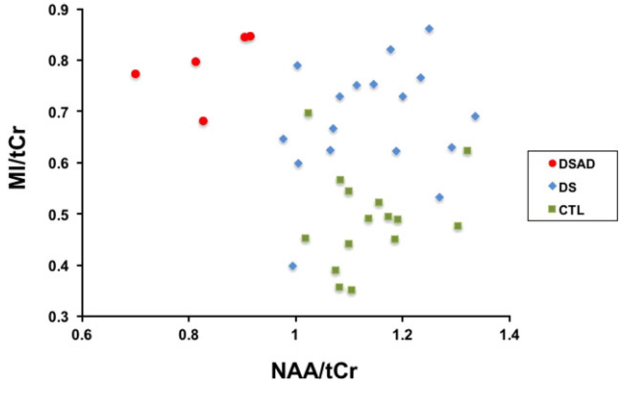

Fig. 3. Plot of NAA/MI ratio as diagnostic criteria for DSAD. Significantly lower NAA and relatively higher MI (i.e. the NAA/MI ratio) separates DSAD from DS and CTL. NAA and MI were normalized to creatine.

Last, we provide novel data showing a link between decreased NAA in the PCC in DS that reflects cognitive functioning as reflected in BPT and SIB scores in DS with and without dementia. In addition, these findings support prior reports showing that the PCC is involved in attentional control (Small et al., 2003) and focus (Leech and Sharp, 2014); PCC has also been linked to constructional ability in early AD (Nobili et al., 2005). The involvement of the PCC in cognition is evolving as new concepts and its associations with the default mode network are being described. For example, reports that are relevant to our current findings in DS, PCC connectivity changes have been reported in Alzheimer's disease (AD) as well as Mild Cognitive Impairment (MCI) along with association with memory performance (e.g. (Zhou et al., 2008)). Further, Leech et al. (2011) and Leech and Sharp (2014) have developed a theoretical framework, of the PCC as an 'information processing hub' given its connectivity to heteromodal association cortex, limbic and paralimbic structures, as well as cognitive functions such as working memory (Leech and Sharp, 2014; Leech et al., 2011). If we apply Leech and colleague's model of the PCC as an 'Arousal, Balance, and Breadth of Attention' model to our present findings, the group differences seen on the BPT and SIB reflect changes in PCC support of cognitive control and memory retrieval as well as multitasking as evaluated by these procedures.

The NAA-MI ratio has been widely considered as sensitive to disease progression and treatment efficacy in AD (Lin et al., 2005). In particular, the NAA/MI ratio discriminates reliably between AD subjects and normal individuals in the general population and provides useful outcomes, as an adjunct to structural MRI and other physiological imaging (Jones and Waldman, 2004; Lin et al., 2012). To further distinguish the neuronal-glial interplay among the three groups, we examined the ratio between NAA and MI as has been reported for sporadic AD (Lin et al., 2005). We found similar results in adults with DS in the present study. Compared to NAA or MI when used alone, the mean NAA/MI ratio was statistically different in the CTL, DS and DSAD diagnostic groups. In particular, the individual variability of this ratio in the five demented DS participants was low, suggesting that the NAA/MI ratio had a high sensitivity to detect pathological and functional status among DS individuals, even with a small sample size. Some caution is warranted regarding the diagnostic use of the NAA/MI ratio based on our sample. Despite 100% correct classification with this group of participants, confidence intervals are broad based on the small sample and the variability seen in the DS only group (Fig. 3). Thus there may be different cutoffs generated across independent studies and it would be interesting to combine results across studies to determine how this cut off generalizes.

Decreased NAA and increased MI have also been observed in the hippocampus of DS adults by MRS (Beacher et al., 2005; Lamar et al., 2011) and in an early report of one individual with DS in the PCC (Shonk et al., 1995). In people with DS with and without dementia, hippocampal measures of Glx did not distinguish these two groups, and neither was different from controls (Tan et al., 2014). In this study, we chose a priori to do the measurements in PCC because PCC demonstrates early metabolic deficits and astrocytic inflammation in AD (Leech and Sharp, 2014; Minoshima et al., 1997) and may provide a more reliable set of measures as it is less sensitive to head movement.

The marked increases of MI in all of the DS participants indicated that this group had higher glial inflammation compared to the healthy controls, which might make them more susceptible to AD. Our results

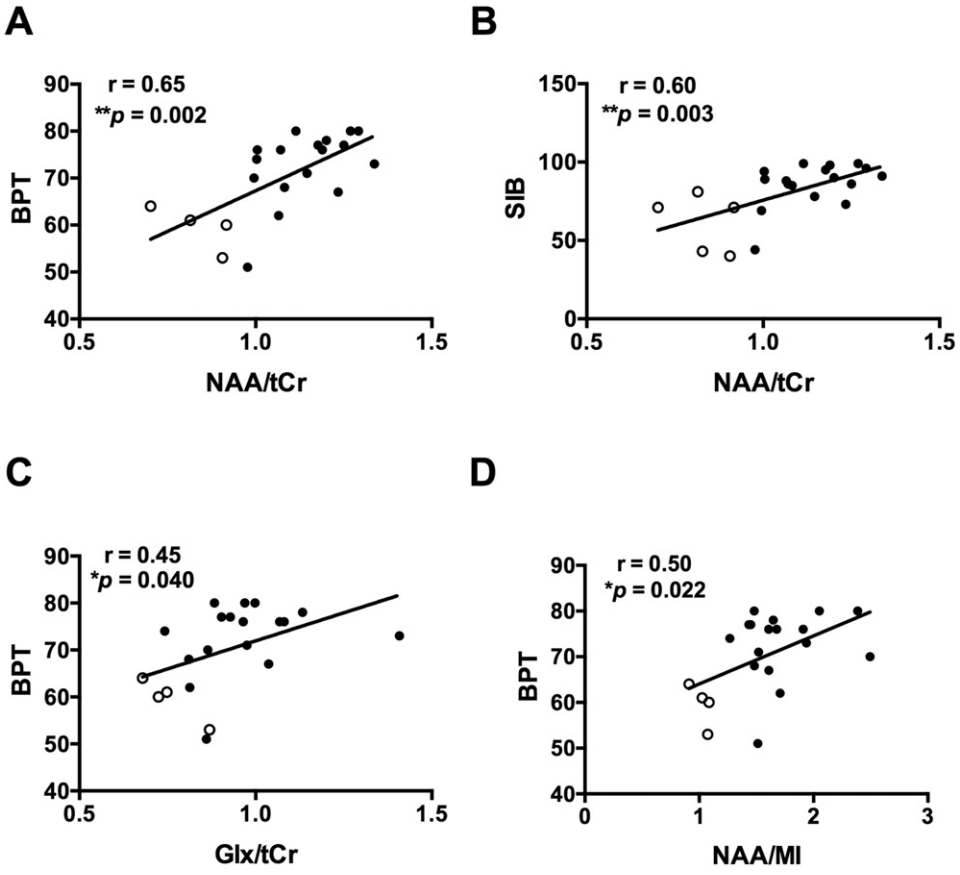

Fig. 4. Positive correlations between brain metabolites and cognitive outcome measures in adults with Down syndrome. NAA was positively correlated with (A) BPT and (B) SIB; BPT was also positively correlated with (C) Glx and (D) NAA/MI ratio. All the metabolites were normalized to creatine. Open circles show the people who were demented and closed circles were nondemented.

are consistent with previous reports of increased MI by MRS in the occipital and parietal cortex (Huang et al., 1999), and hippocampus (Beacher et al., 2005) in nondemented adults with DS. Lamar et al. (2011) also found that hippocampal MI by MRS was higher in demented adults with DS compared to those without dementia (Lamar et al., 2011). In a description of one individual with DS, MRS of the PCC also showed increased MI (Shonk and Ross, 1995). Further, MI levels in the PCC are correlated with cognitive scores. It is interesting to note that the MI cotransporter (SLC5A3) gene is on chromosome 21 and overexpressed in DS (Berry et al., 1995) and further, that synaptojanin 1, which is also overexpressed in DS, leads to increased gliosis (Herrera et al., 2009). Thus, higher MI levels in DS may reflect developmental differences in DS, with a lesser involvement in AD pathogenesis per se. Consistent with this interpretation are the relatively weak correlations between MI and cognitive measures that reflect dementia in the current study, as well as a study in 3–15 year old children with DS showing similar decreased mI/Cr ratios (Smigielska-Kuzia et al., 2010).

We recognize that there may be a systematic difference in brain volume and composition between our demented and nondemented participants. A recent study suggests that partial volume effects are important when quantifying MRS and that knowing the voxel composition of grey and white matter as well as cerebrospinal fluid can reduce variability in studies that include people with neurodegenerative diseases (Mato Abad et al., 2014) Further, there is an age related decrease in cortical thickness in the cingulate gyrus in nondemented adults with DS, which is more rapid between 20–30 years of age (Romano et al., 2016). This suggests that decreased NAA in our study may reflect partial volume effects and potentially be overestimated On the other hand, MI increases, against a potential decrease in volume and thus may be a conservative estimate. In the current study, there were delays in the time between anatomical imaging and MRS to reduce the time participants were required to be in the scanner, which can be a challenge for people with DS. In ongoing studies, we are now ensuring that these two imaging protocols are acquired together.

Brain metabolism is tightly coupled with cerebrovascular function (Lin et al., 2010; Fox et al., 1988) and brain hypoperfusion in DS adults (Gupta and Ratnam, 2011). It is interesting to note that people with DS appear to be protected from some cerebrovascular risk factors including being relatively free of atherosclerosis (Murdoch et al., 1977) and less frequent hypertension (Draheim et al., 2010; Draheim et al., 2002; Morrison et al., 1996).However, there is extensive cerebral amyloid angiopathy in DS brain (Belza and Urich, 1986; Ikeda et al., 1994) and this may lead to microhemorrhages and strokes (Belza and Urich, 1986; Donahue et al., 1998; Jastrzebski et al., 2015). Not all studies report strokes in the aging brains of people with DS (Ikeda et al., 1994; Lai and Williams, 1989). In older adults with DS who were nondemented, hyoperfusion is observed in the PCC (Haier et al., 2003), the temporal and frontal cortices as well as the hippocampus (Haier et al., 2008).It is important for future studies to investigate the role of cerebrovascular dysfunction and the development of dementia in DS using non-invasive, well-validated neuroimaging methods, including cerebral blood flow and cerebral blood volume measurements (Wilcock et al., 2015b).

5. Conclusions

In conclusion, we used ^{1}H-MRS to identify metabolic deficits as surrogate markers of dementia in adults with DS. Novel features of our study include the systematic imaging of the posterior cingulate cortex in a cohort of adults with DS, which is vulnerable to early AD

neuropathology and the correlation with cognitive test scores. Although we assume that AD in DS is similar to sporadic AD, the current study confirms this hypothesis with respect to MRS biomarkers in the posterior cingulate. Caveats to our study include the small sample size of adults with dementia with DS and the need for longitudinal measures. In future it will be useful to the current data to establish a ROC that can be tested in additional participants as they are recruited to the study. We are currently following the described DS groups in a longitudinal study of aging in DS. The current imaging criteria require replication in a second cohort but may have future clinical implications for DS individuals, such as aiding early detection of risk for dementia, longitudinal follow-up of metabolic changes, and evaluation of therapeutic efficacy.

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