HHS Public Access

Author manuscript

J Neurovirol. Author manuscript; available in PMC 2015 September 04.

Published in final edited form as:

J Neurovirol. 2010 November ; 16(6): 435–444. doi:10.3109/13550284.2010.520817.

Cerebral metabolite abnormalities in human immunodeficiency virus are associated with cortical and subcortical volumes

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Abstract

Cerebral metabolite disturbances occur among human immunodeficiency virus (HIV)-infected people, and are thought to reflect neuropathology, including proinflammatory processes, and neuronal loss. HIV-associated cortical atrophy continues to occur, though its basis is not well understood, and the relationship of cerebral metabolic disturbance to structural brain abnormalities in HIV has not been well delineated. We hypothesized that metabolite disturbances would be associated with reduced cortical and subcortical volumes. Cerebral volumes were measured in 67 HIV-infected people, including 10 people with mild dementia (acquired immunodeficiency syndrome [AIDS] dimentia complex [ADC] stage >1) via automated magnetic resonance imaging (MRI) segmentation. Magnetic resonance spectroscopy (MRS) was used to measure levels of

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Declaration of interest: The authors report no conflicts of interest.

cerebral metabolites *N*-acetylaspartate (NAA), *myo*-inositol (MI), choline-containing compounds (Cho), glutamate/glutamine (Glx), and creatine (Cr) from three brain regions (frontal gray matter, frontal white matter, basal ganglia). Analyses were conducted to examine the associations between MRS and cerebral volumetric measures using both absolute and relative metabolite concentrations. NAA in the mid-frontal gray matter was most consistently associated with cortical (global, frontal, and parietal), ventricular, and caudate volumes based on analysis of absolute metabolite levels, whereas temporal lobe volume was associated with basal ganglia NAA and Glx, and Cho concentrations in the frontal cortex and basal ganglia. Hippocampal volume was associated with frontal white matter NAA, whereas thalamic volume was associated with both frontal white matter NAA and basal ganglia Glx. Analyses of relative metabolite concentrations (referenced to Cr) yielded weaker effects, although more metabolites were retained as significant predictors in the models than the analysis of absolute concentrations. These findings demonstrate that reduced cortical and subcortical volumes, which have been previously found to be linked to HIV status and history, are also strongly associated with the degree of cerebral metabolite disturbance observed via MRS. Reduced cortical and hippocampal volumes were most strongly associated with decreased NAA, though reduced Glx also tended to be associated with reduced cortical and subcortical volumes (caudate and thalamus) as well, suggesting both neuronal and glial disturbances. Interestingly, metabolite-volumetric relationships were not limited to the cortical region from which MRS was measured, possibly reflecting shared pathophysiological processes. The relationships between Cho and volumetric measures suggest a complicated relationship possibly related to the effects of inflammatory processes on brain volume. The findings demonstrate the relationship between MRI-derived measures of cerebral metabolite disturbances and structural brain integrity, which has implication in understanding HIV-associated neuropathological mechanisms.

Keywords

brain; cerebral metabolites; cortical volume; HIV; magnetic resonance spectroscopy; morphometry; subcortical volume

Introduction

Human immunodeficiency virus (HIV) infection continues to cause cognitive impairments and brain dysfunction despite the fact that highly active antiretroviral therapy (HAART) has dramatically reduced mortality and the prevalence of HIV-associated dementia (Sacktor *et al*, 2002). Clinical and neuropathological studies suggest that HIV will continue to exert detrimental brain effects even when viral load is well controlled (Sacktor *et al*, 2002; Brew, 2004; McArthur, 2004; Valcour and Sacktor, 2002).

Structural brain abnormalities have been reported since the early years of the HIV epidemic, with evidence of reduced cortical volumes, particularly among people with advanced disease in the pre-HAART era (Aylward *et al*, 1995; Heindel *et al*, 1994; Jernigan *et al*, 1993). Following the advent of HAART, cortical brain changes received less attention, as it appeared that antiretroviral therapies were ameliorating many of the brain disturbances associated with HIV. Yet recent studies suggest that structural brain abnormalities continue

to occur, particularly of the basal ganglia and cerebral white matter. For example, reduced white matter integrity has been found among a cohort of largely HAART-treated HIV-infected individuals compared to healthy controls (Gongvatana *et al*, 2009). Relatively few cortical volumetric studies exist in the post-HAART era, though emerging evidence suggests that cortical atrophy continues to occur (Thompson *et al*, 2005). We recently observed significant relationships between clinical variables and both cortical and subcortical volumes among people infected with HIV, with disease history (nadir CD4, duration of infection) being most strongly associated with cortical volumes (Cohen *et al*, 2010). This raises the concern that brain pathology incurred during the clinical history may increase vulnerability to neurodegeneration, with cortical changes analogous to premature aging (Brew *et al*, 2009; Valcour *et al*, 2004).

A separate line of research has focused on characterizing cerebral metabolite disturbances among patients with HIV, in an effort to link the viral and immunological effects of HIV with neuropathological processes in the brain. Proton (^{1}H) magnetic resonance spectroscopy (MRS) provides measures of specific metabolites that reflect different pathological processes. Numerous MRS studies of HIV have demonstrated abnormalities of the cerebral metabolites *N*-acetylaspartate (NAA), *myo*-inositol (MI), choline-containing compounds (Cho), and glutamate/glutamine (Glx) (Chang *et al*, 2004; Schifitto *et al*, 2009a; Schweinsburg *et al*, 2005; Paul *et al*, 2007; Lentz *et al*, 2009). Decreased NAA is known to reflect neuronal injury or loss, whereas increased Cho and MI have been linked to proinflammatory processes, and a breakdown of cell membranes (Ross and Bluml, 2001). Glx abnormalities have also been observed among patients with HIV, though the neuropathological implications are unclear. Furthermore, there is mounting evidence that cerebral metabolite abnormalities detected on MRS are associated with impaired neurocognitive function and that they are greatest among people with HIV-associated dementia (Chang *et al*, 2004; Paul *et al*, 2007, 2008; Patel *et al*, 2003; Yiannoutsos *et al*, 2004). Yet, the relationship of these cerebral metabolites to structural brain abnormalities in HIV has not been well delineated, and a full understanding of the evolution of cortical and subcortical injury in the context of chronic infection is still lacking.

To address this question, we examined the relationship between cerebral metabolite concentrations measured by MRS and the volumes of specific cortical and subcortical regions. Structural magnetic resonance imaging (MRI) and MRS data were obtained from a patient pool of HIV-infected patients followed in a large multicenter study designed to assess the effects of HIV infection on brain structure and function. The extent to which HIV clinical factors together with cerebral metabolite abnormalities contributed to association with brain volumes was also assessed. We hypothesized that reduced cortical and subcortical volumes would be significantly associated with the extent of metabolite disturbance.

Results

Table 1 summarizes the demographic and clinical characteristics of the sample.

Two sets of analyses were performed with the MRS metabolites as predictors in the regression models. Absolute MRS measures were used in the first set of analyses, whereas

the second set of analyses used metabolite/creatine (Cr) ratios. Table 2 shows the relationships between regional brain volumes and cerebral metabolite measures.

Absolute metabolite concentration analyses

Global brain volumes—Increased total gray matter volume was associated with increased NAA in the frontal gray matter ($P < .0001$). Increased ventricle volume was related to decreased NAA in the frontal gray matter ($P < .0001$), decreased NAA in the basal ganglia ($P = .0265$), and increased Glx in the frontal white matter ($P = .072$). There were no associations found between the metabolite levels and total white matter volume.

Cortical regional volumes—Increased frontal lobe volume was associated with increased NAA in the frontal gray matter ($P < .0001$). Increased temporal lobe volume was associated with increased NAA ($P < .0001$), decreased Cho ($P = .0031$), and increased Glx ($P = .0168$) in the basal ganglia; and increased Cho in the frontal gray matter ($P = .0012$). Increased parietal lobe volume was related to increased NAA in the frontal gray matter ($P = .0001$) and decreased Glx in the frontal white matter ($P = .029$).

Subcortical structures and the hippocampus—Increased caudate volume was associated with increased NAA ($P = .0083$) and a trend towards increased Glx ($P = .069$) in the frontal gray matter. Increased thalamic volume was associated with increased NAA in the frontal white matter ($P = .001$), and increased Glx in the basal ganglia ($P = .0021$). Increased hippocampal volume was associated with increased NAA in the frontal white matter ($P = .0025$).

Relative metabolite concentration analyses

Global brain volumes—Increased total gray matter volume was associated with increased Cho in the frontal gray matter ($P = .0081$), decreased Glx in the frontal white matter ($P = .0131$), and increased NAA ($P = .0311$) and decreased Cho ($P = .0359$) in the basal ganglia. Increased ventricular volume was related to decreased NAA ($P = .0083$) and increased Cho ($P = .0466$) in the basal ganglia, decreased NAA ($P = .0268$) and increased Glx ($P = .0094$) in the frontal white matter, and decreased Cho in the frontal gray matter ($P = .0065$). Again there were no associations found between the metabolite levels and total white matter volume.

Cortical regional volumes—Increased frontal lobe volume was associated with increased NAA ($P = .0108$) and decreased Cho ($P = .0403$) in the basal ganglia, increased NAA ($P = .0447$) and decreased Glx ($P = .0085$) in the frontal white matter, and increased Cho in the frontal gray matter ($P = .0022$).

Increased temporal lobe volume was associated with increased NAA ($P = .0001$) and decreased Cho ($P = .0001$) in the basal ganglia, and increased Cho in the frontal gray matter ($P < .0001$). Increased parietal lobe volume was associated with increased NAA in the frontal gray matter ($P = .006$) and decreased Glx in the frontal white matter ($P = .0016$).

Subcortical structures—Only the thalamus was found be significantly associated with relative concentrations of the MRS metabolites. Increased thalamic volume was associated with increased NAA (P = .0124) in the frontal white matter, and increased Cho (P = .0434) and Glx (P = .0742) in the frontal gray matter.

Contribution of clinical measures

The addition of the clinical variables to the models did not change the MRS indices retained as the best predictors of the brain volumes. Furthermore, these additional variables were retained as significant predictors in only a few of the analyses. None of the clinical variables were retained as significant predictors for analyses conducted with relative metabolite concentrations. When absolute metabolite concentrations were analyzed, clinical variables were retained as predictors for total cortical gray matter, frontal gray matter, and caudate nucleus. Longer duration of HIV infection was associated with decreased total cortical gray matter volume (P = .0434), though the addition of this variable did not substantially increase the overall strength of the model (adjusted R^2 = .2431, P = .0013). Participants with undetectable plasma HIV viral load exhibited increased frontal lobe volume (P = .0385), and the addition of this variable resulted in a 4.2% increase in the strength of the model (adjusted R^2 = .3442, P < .0001). Undetectable HIV viral load was also associated with increased caudate volume (P = .0029), resulting in a 5.4% increase in model strength (adjusted R^2 = .3234, P < .0001). The clinical indices were not retained as significant predictors of any other brain volumetric measure. Neither acquired immunodeficiency syndrome (AIDS) dementia complex (ADC) stage nor the interaction of ADC stage with any MRS metabolite measure was retained as significant predictors of any of the brain volumes.

Discussion

Initial optimism that HIV-associated structural brain abnormalities would be relatively rare following the widespread use of HAART have been tempered by recent evidence. Data from our group recently demonstrated reduced brain volumes in HIV-infected people relative to age-matched seronegative controls (Tate *et al*, 2010). Another recent study in the same cohort showed that cortical and subcortical volumes were significantly associated with HIV disease history and current clinical status (Cohen *et al*, 2010). In the current study, we extended these findings by demonstrating the link between cortical and subcortical brain volumes derived from structural MRI and cerebral metabolite measures derived from MR spectroscopy reflecting neuronal damage, cell membrane breakdown, inflammatory pathophysiology, and neurotoxicity. It is noteworthy that these findings were evident in the context of well-controlled HIV disease, as only about a third of the cohort exhibited clinically significant cognitive problems (i.e., ADC stage ≥0.5).

The most robust findings in this study involve the relationship between NAA and absolute metabolites concentrations. Cortical NAA was positively associated with both total cortical gray matter volume, and the volumes of specific cortical regions including the frontal and parietal cortex. Furthermore, decreased NAA levels in both the mid-frontal gray matter and basal ganglia were associated with increased ventricular volume. These findings are consistent with the idea that decreased NAA is an indicator of neuronal damage or loss.

Glx concentrations also emerged as significant predictors of many cortical and subcortical volumes. Though the direction of the relationships differed across regions, Glx tended to be negatively associated with cortical volume and positively associated with ventricular volume. These findings are consistent with previously reported associations between increased Glx and brain damage caused by chronic and acute hepatic encephalopathy, hypoxia, near drowning, and ornithine transcarbamylase deficiency (Binesh *et al*, 2006; Gropman and Batshaw, 2004; Kreis *et al*, 1996; Long *et al*, 2009; Sijens *et al*, 2008). Findings for other brain disorders have been mixed with regards to the direction of Glx effects. For example, reduced Glx concentrations have been observed in Alzheimer's disease (Antuono *et al*, 2001; Jones and Waldman, 2004). In HIV, human and animal models have shown decreased levels of cerebral Glx (Lentz *et al*, 2009; Ratai *et al*, 2009; Chang *et al*, 1997), including patients with progressive multifocal leukoencephalopathy. Another study showed decreased frontal Glx concentrations, along with increased white matter integrity on diffusion tensor imaging among patients treated with lithium for HIV-associated cognitive impairment (Schifitto *et al*, 2009b). Accordingly, although alteration of cerebral Glx level appears to be common in brain disorders, whether increased or decreased levels are observed seems to depend on the nature of the underlying disturbance. Additional studies are needed to better understand Glx effects in HIV, particularly in the context of antiretroviral therapy and comorbidities such as hepatitis C, liver disease, and substance abuse.

The fact that increased Cho in the frontal gray matter was related to increase in overall cortical, frontal, and temporal gray matter volumes, and decreased ventricular volume was somewhat unexpected; as one might assume that the pathophysiology reflected by elevated Cho would also manifest itself through volume loss. Although the reason for this effect cannot be determined from this study, this finding suggests that inflammatory processes linked to Cho may actually contribute to an increase in brain volume, perhaps as a result of swelling and cellular responses to injury. Interestingly, the opposite effect was observed for Cho in the basal ganglia, as increased basal ganglia Cho level correlated with decreased cortical volumes and increased ventricular volume. The fact that the direction of effect for Cho depended on whether it was measured from the cortical gray matter versus basal ganglia suggests that the neuropathology (e.g., inflammation) associated with Cho may differ across different of brain tissues. The fact that different directions of Cho effects occurred within single statistical models for particular cortical areas suggests that the relationship between cerebral Cho levels and brain volume may be more complicated than for other metabolites.

MI failed to be retained as significant correlate of cortical or subcortical volumes in any of the statistical models. Cerebral MI levels have been linked to HIV status and history in past studies (Chang *et al*, 1999, 2002, 2003, 2004; Yiannoutsos *et al*, 2004; Sacktor *et al*, 2005; Tarasow *et al*, 2003). There is evidence that MI levels are associated with gliosis and inflammatory response (Yiannoutsos *et al*, 2004; Jones et al, 2004; Broom *et al*, 2007; Tkac *et al*, 2007). That it was not related to brain volume is noteworthy, though the meaning of this is not entirely clear at this point.

Interestingly, temporal lobe volume did not relate to metabolite concentrations in the same manner as other cortical regions. Whereas frontal and parietal volumes were most strongly associated with NAA levels in the mid-frontal gray matter, temporal lobe volume was more strongly associated with NAA, Cho, and Glx levels in the basal ganglia, and Cho level in frontal grey matter. The fact that hippocampal volume was associated with NAA level in the frontal white matter, and not grey matter, also indicates that temporal/hippocampal brain volumes may be differently affected than other regions.

It is noteworthy that the observed metabolite-volumetric relationships occurred in regions that were not confined to regions where MRS was sampled. For example, parietal lobe volume was associated with NAA level in the frontal gray matter; frontal lobe volume was not only associated with NAA level in the frontal lobe, but also in the basal ganglia; and temporal lobe volume was associated with basal ganglia NAA level. These results suggest that metabolite concentrations in a specific brain region can be sensitive to changes occurring more globally across the cortex, likely reflecting interrelated pathophysiological processes.

Comparison of the results derived from analyses of the absolute versus relative metabolite concentrations provides potentially useful insights into the findings from cerebral MRS studies in HIV. The magnitudes of the metabolite-volumetric relationships based on analysis of the absolute metabolite concentrations were generally greater. Furthermore, the analysis of absolute metabolite concentrations tended to point to the dominance of fewer metabolites being associated with brain volumes. In contrast, the analysis of relative metabolite concentrations yielded weaker overall magnitude of associations, but with a larger number of metabolites contributing to the association. For example, frontal lobe volume was strongly associated only with frontal gray matter NAA absolute concentrations with an overall model R^2 of .30; but was related to NAA/Cr, Cho/Cr, and Glx/Cr measures in the frontal grey matter, frontal white matter, and basal ganglia with a significantly lower overall model R^2 of .22. Frontal gray matter NAA typically did not emerge as a significant predictor in the relative concentration models. This was likely a direct result of the significant correlation between frontal gray matter NAA and Cr ($R = .75$, $P < .05$), thus diminishing the influence of the NAA/Cr ratio as a predictor. The implication of these findings is that relative concentrations may be useful for detecting smaller effects of Cho and Glx on brain volume in HIV, whereas absolute concentrations may provide a better estimate of metabolites account for the majority of volumetric effects.

The fact that few clinical variables were retained along with the metabolite levels as significant predictors of brain volume is also noteworthy. When the clinical variables are considered independent of the MRS measures, they are predictive of brain volumes (Cohen *et al*, 2010). Yet, these effects are relatively small compared to the association between cerebral metabolite concentrations and brain volumes, and the current findings indicate that when both types of data are considered together, the MRS measures are better at predicting both cortical and subcortical volumes. This is not surprising given the fact that the both cerebral metabolite and volumetric indices are both direct brain measures, whereas the clinical indices less directly reflect brain pathology. Furthermore, most patients in the study were neuroasymptomatic, HAART-treated, and had relatively well-controlled HIV disease.

Among the few clinical variables that emerged, undetectable HIV viral load was significantly associated with increased frontal and caudate volumes, indicating the importance of virologic control. Duration of infection was related to decreased total cortical gray matter volume, pointing to the impact of disease history (Cohen *et al*, 2010). The lack of effects of ADC stage and its interaction with metabolite concentrations likely reflects the low rate of neurocognitive disorder in the cohort (85% with ADC stage <1). Importantly these findings also suggest that the association between cerebral metabolite concentrations and brain volume may exist independent of cognitive and functional status.

The current results demonstrated that clear relationships exist between cerebral metabolite levels and the cortical/subcortical brain volumes in HIV-infected individuals despite effective antiretroviral treatment. This reinforces previous findings that HIV-infected patients in the HAART era are still affected by brain disturbances. However, it remains unclear whether this reflects ongoing neuropathology associated with chronic HIV infection, or damage sustained at some point in the past, perhaps before viral load was adequately controlled with medication. Typically, gross morphometric changes are thought to occur as a late manifestation of neurodegenerative condition, such as Alzheimer's disease. Yet, in the current study, relationships between the cerebral metabolites measured by MRS and brain volume were observed among patients who for the most part were asymptomatic and have good immune function and virologic control. In light of our previous findings that nadir CD4 and duration of infection are among the clinical measures most strongly associated with brain volumes in HIV-infected people (Cohen *et al*, 2010), it is possible that factors tied to patients history of infection are playing a significant role in affecting brain structure beyond current disease status.

The differential susceptibility regions of the brain to volume loss and their relationship to cerebral metabolite levels measured by MRS points to the potential power of these methods for studying and measuring the effects of host and viral factors as well as their interactions, and together provide a framework to further understand structural change in the HIV infected brain in the setting of chronic disease and HAART. It should be emphasized that these findings are based on cross-sectional analysis of baseline data. Validation of these results will depend on longitudinal studies integrating multimodal neuroimaging data, including MRS and morphometry, along with neurocognitive and clinical measures. The relationship between neuronal loss and the more acute pathologiscal processes associated with HIV on brain structure needs to be disentangled in future studies.

Methods

Clinical sample

Sixty-seven HIV-infected patients enrolled in a longitudinal study of HIV were assessed as part of their baseline examinations. The sample had a larger proportion of men (82%) than women. Patients came from HIV clinics at three sites (University of Rochester, University of Colorado, and Stanford University) and consisted of the first set of individuals for whom brain morphometry data were available. Table 1 summarizes the demographic and clinical characteristics of the sample.

This clinical cohort consisted of individuals who were middle-aged (mean = 48.1, SD = 7.8 years) and relatively well educated (63% with some college or more education). Most had been infected for over 10 years (66%) and had a CD4 nadir <50 cells/ml (59%). A majority (81%) of participants were HAART-treated at the time of the study. Accordingly, we observed more intact current CD4 levels (on the $\log_{10}$ scale, mean = 2.52, SD = 0.26), and only a small proportion (18%) of participants with detectable plasma viral load.

Neurocognitive status was assessed and using the AIDS dementia complex staging scale, as previously described (Brew *et al*, 1995). Patients were assessed on both clinical and neuropsychological tests and rated as no impairment, stage 0; subclinical impairment, stage 0.5; mild impairment, stage 1; moderate, stage 2; or severe, stage 3. Neurocognitive impairment was defined as performance of at least 1.0 standard deviation below normative values on two or more neuropsychological tests or at least 2.0 standard deviations below normative values on one or more tests within any neurocognitive domains. Subsequent to the inception of this study, Antinori and colleagues published a new classification in which ADC stages 0.5 and 1 would correspond to mild cognitive disorder or motor neuron disease (MND) and ADC stage 2 or greater would correspond to HIV-associated dementia or HAD (Antinori *et al*, 2007). Ten participants (17%) currently exhibited at least mild AIDS dementia complex (ADC stage ≥1), whereas the remaining 57 individuals were neuroasymptomatic (NA).

Structural MRI data acquisition and processing

High-resolution whole-brain structural images were acquired using a T1-weighted MPRAGE sequence with the following parameters: TE = 3.57 ms, TR = 2730 ms, flip angle = 7°, FOV = 256 × 256 mm, 1 × 1 × 1 mm resolution. Brain volumes were measured with the Individual Brain Atlases using Statistical Parametric Mapping IBASPM (Alemán-Gómez *et al*, 2006), toolbox for the Statistical Parametric Mapping 5 (SPM5; Wellcome Department of Imaging Science; www.fil.ion.ucl.ac.uk/spm/) software package running under Matlab. Image processing involved segmentation of individual brain volumes into gray matter, white matter, and cerebrospinal fluid (CSF) compartments, yielding estimated volumes for each compartment, along with the total intracranial volume. Segmented brain volumes were normalized via nonlinear registration to the MNI152 template, and gray matter voxels were labeled according to a predefined anatomical atlas (Tzourio-Mazoyer *et al*, 2002). Labeled brain volumes were then inverse-transformed into their native spaces, yielding estimated volumes of specific brain regions according to the atlas labels. To correct for head size variability, ratios of gray matter, white matter, and ventricular volumes, and lobular volumes to total intracranial volume were used as volumetric measures.

MRS data acquisition and processing

Single-voxel ^{1}H spectra were acquired using a customized version of the PRESS sequence. Voxels 6 cc in volume were prescribed in three regions: midline frontal gray matter, right or left frontal white matter in the centrum semiovale, and right or left basal ganglia. The hemisphere for frontal white matter and basal ganglia regions was alternated from subject to subject. Field homogeneity and water suppression were adjusted using automated algorithms

from GE and Siemens. Water suppressed spectra were collected with TE/TR = 35/3000 ms, bandwidth = 2500 Hz, 128 averages, NEX = 8.

In addition, the customized pulse program automatically collects single-scan fully relaxed water FIDs from each voxel at 7 different echo times (TE = 30, 45, 65,100, 200, 500, and 1500 ms; TR = 15 s) from which metabolite concentrations are calculated. To control for a possible instrument bias, we collected phantom MRS data concurrently with the subject evaluation using the identical protocol as described above. The metabolite levels obtained from the phantoms provide a summary of conditions present at the time of the subject evaluation thus explaining some of the variations across sites and time points.

The time domain spectral data were transferred to an FTP server at the central MRS processing site Hawaii. The metabolite ratios NAA/Cr, Cho/Cr, MI/Cr, and Glx (= Glu + Gln)/Cr were determined using the LC Model spectral analysis software (Provencher, 2001) and an unsuppressed water FID at TE = 30 ms for eddy-current correction. This technique yields interindividual variations of about 10% to 15%, and intrasubject variability of 3% to 8%, for the concentrations of the major metabolite peaks (Lee *et al*, 2003).

Statistical analysis

We tested whether the MRS metabolites (NAA, Cho, MI, Glx) measured in three brain regions (frontal gray matter, frontal white matter, and basal ganglia) were differentially associated with brain volumes using linear regression. Three sets of volumetric measures were used as dependent variables: (1) gray matter, white matter, and total ventricular volumes; (2) frontal, parietal, and temporal lobe cortical volumes; and (3) caudate, putamen, thalamus, and hippocampus volumes. The MRS metabolites serving as the independent measures were analyzed using two different methods: (1) absolute metabolite concentrations; (2) ratios of metabolite/Cr. The rationale using both of these approaches is that clinical research literatures in HIV now exist for MRS using both absolute and relative metabolite concentrations. Subsequently, HIV clinical variables (current CD4, nadir CD4, plasma HIV RNA, years since HIV diagnosis, antiretroviral treatment status), hepatitis C virus (HCV) status, ADC stage were entered into the regression analyses to examine the added value of these variables in predicting brain volumes.

All linear regression models were adjusted for age, sex, and race. All statistical analyses were done using R-2.9.2 (R Core Development System: http://www.rproject.org). Final linear regression models were selected by minimizing Akaike information criterion (AIC) (Akaike, 1974; Burnham and Anderson, 2002), which balances the model fit and its complexity. Increasing the number of parameters in the models improves their fit to the data, but at a cost of increased complexity. AIC balances the goodness of fit and the number of included covariates by penalizing the number of parameters in the model. The best model is the one with the lowest AIC. This method is more robust than the traditional stepwise selection procedures and produces parsimonious models balancing the goodness of fit and model complexity. To ameliorate the selection of models that might contain non-significant variables, we used a bootstrap procedure on the results of the initial fit, and only chose the variables for the final models that were selected in more than 70% of the bootstrapped samples.

Acknowledgments

The authors would like to acknowledge and express their appreciation for the efforts on all the MRS consortium sites involved in this multicenter project, including the Colorado Clinical and Translational Research Institute (RR025780, Sokol, RJ), Principal Investigator [PI], and the staff, clinicians, and investigators from these sites that made this study possible. The authors also appreciate the support of the National Institutes of Health who provide the support and funding for this research, including R01 NS036524 “Proton MRS Studies of Cerebral Injury in HIV Infection” (Brad Navia, PI); R01 MH074368 “Age Effects on HIV-Associated Brain Dysfunction” (Ronald Cohen, PI).

The authors alone are responsible for the content and writing of the paper.

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Table 1

Demographic and clinical characteristics of participants in the AIDS dementia complex (ADC) groups (continuous variables reported as mean ± SD, and categorical variables as percentages)

	ADC stage		
	0 and 0.5	**≥1**	**Total**
N	57	10	67
Gender (% male)	85	80	82
Age (years)	48.0 ± 8.2	48.7 ± 5.9	48.1 ± 7.8
Ethnicity (% Caucasian)	74	70	73
Education level (% some college or more)	60	80	63
% IV drug use	19	40	22
Duration of infection (years)	11.5 ± 5.8	16.0 ± 5.4	12.2 ± 5.9
CD4 count (log_{10} cells/ml)	2.55 ± 0.22	2.38 ± 0.43	2.52 ± 0.26
Nadir CD4 (log_{10} cells/ml)	1.48 ± 0.51	1.33 ± 0.38	1.46 ± 0.50
% undetectable Plasma RNA viral load	82	80	82
% known on ART at baseline (uninterrupted)	84	60	81

Note. The two ADC groups did not significantly differ on any demographic or clinical variables.

J Neurovirol. Author manuscript; available in PMC 2015 September 04.

Table 2

Relationships between regional brain volumes and cerebral metabolites

Absolute metabolite measures					Metabolite/Cr ratios				
	β	*P*	Adjusted R^2	*P*		β	*P*	Adjusted R^2	*P*
Gray matter volume			.2396	.0003	Gray matter volume			.1580	.0147
NAA in FGM	.0158	<.0001			Cho in FGM	.3078	.0081		
					Glx in FWM	−.0445	.0131		
					NAA in BG	.0430	.0311		
					Cho in BG	−.2730	.0359		
Ventricular volume			.4335	<.0001	Ventricular volume			.2184	.0035
NAA in FGM	−.0240	<.0001			Cho in FGM	−.4392	.0065		
NAA in BG	−.0108	.0265			NAA in BG	−.0751	.0083		
Glx in FWM	.0069	.0720			Glx in FWM	.0651	.0094		
					NAA in FWM	−.0728	.0268		
					Cho in BG	.3575	.0466		
Frontal lobes volume			.3020	<.0001	Frontal lobes volume			.2202	.0034
NAA in FGM	.0062	<.0001			Cho in FGM	.1150	.0022		
					Glx in FWM	−.0152	.0085		
					NAA in BG	.0167	.0108		
					Cho in BG	−.0851	.0403		
					NAA in FWM	.0152	.0447		
Temporal lobes volume			.5404	<.0001	Temporal lobes volume			.3942	<.0001
NAA in BG	.0033	<.0001			Cho in FGM	.0798	<.0001		
Cho in FGM	.0080	.0012			NAA in BG	.0121	.0001		
Cho in BG	−.0100	.0031			Cho in BG	−.0780	.0001		
Glx in BG	.0010	.0168							
Parietal lobes volume			.3255	<.0001	Parietal lobes volume			.2755	.0001
NAA in FGM	.0023	.0001			Glx in FWM	−.0083	.0016		
Glx in FWM	−.0009	.0290			NAA in FGM	.0111	0.0060		
Thalamus volume			.3089	<.0001	Thalamus volume			.2662	.0003
NAA in FWM	.4802	.0010			NAA in FWM	1.9652	.0124		

Absolute metabolite measures					Metabolite/Cr ratios				
	β	*P*	Adjusted R^2	*P*		β	*P*	Adjusted R^2	*P*
Glx in BG	.2670	.0021			Cho in FGM	7.1133	.0434		
					Glx in FGM	.9514	.0742		
Caudate volume			.2687	.0002					
NAA in FGM	.4323	.0083							
Glx in FGM	.2175	.0690							
Hippocampus volume			.1694	.0036					
NAA in FWM	.3821	.0025							

Note. Both absolute metabolite measures and metabolite/creatine ratios are included. Volumetric measures have been adjusted by total intracranial volume. Adjusted R^2 values are for the whole model fit.