CONCISE COMMUNICATION

Brain *N*-acetyl-aspartyl-glutamate is associated with cognitive function in older virally suppressed people with HIV

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Objectives: Cognitive impairment persists in virally suppressed people with HIV (VS-PWH) especially in higher order domains. One cortical circuit, linked to these domains, is regulated by *N*-acetyl-aspartyl glutamate (NAAG), the endogenous agonist of the metabotropic glutamate receptor 3. The enzyme glutamate carboxypeptidase II (GCPII) catabolizes NAAG and is upregulated in aging and disease. Inhibition of GCPII increases brain NAAG and improves learning and memory in rodent and primate models.

Design: As higher order cognitive impairment is present in VS-PWH, and NAAG has not been investigated in earlier magnetic resonance spectroscopy studies (MRS), we investigated if brain NAAG levels measured by MRS were associated with cognitive function.

Methods: We conducted a retrospective analysis of 7-Tesla MRS data from a previously published study on cognition in older VS-PWH. The original study did not separately quantify NAAG, therefore, work for this report focused on relationships between regional NAAG levels in frontal white matter (FWM), left hippocampus, left basal ganglia and domain-specific cognitive performance in 40 VS-PWH after adjusting for confounds. Participants were older than 50 years, negative for affective and neurologic disorders, and had no prior 3-month psychoactive-substance use.

Results: Higher NAAG levels in FWM were associated with better attention/working memory. Higher left basal ganglia NAAG related to better verbal fluency. There was a positive relationship between hippocampal NAAG and executive function which lost significance after correction for confounds.

Conclusion: These data suggest brain NAAG serves as a biomarker of cognition in VS-PWH. Pharmacological modulation of brain NAAG warrants investigation as a therapeutic approach for cognitive deficits in VS-PWH.

Graphical abstract: http://links.lww.com/QAD/D137

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Introduction

Combination antiretroviral therapy (cART) has improved and extended the lives of people with HIV (PWH) [1]; however, comorbid cognitive impairment persists affecting up to 40% of individuals [2–4]. As larger proportions of individuals with HIV reach ages over 50 years, the risk for cognitive impairment is greater, especially given accelerated aging in the frontal cortex and hippocampus [5–7]. HIV involves continuous seeding of the brain with infected monocytes and lymphocytes from the periphery [8], resulting in neurotoxicity and neuroinflammation [9–12]. Additionally, though continued volume loss seems to be stopped by treatment with cART, there is evidence HIV infection is associated with reduced cortical volumes [13]. Glutamate excitotoxicity is hypothesized to play a role [14,15]. Attempts to ameliorate hyper-glutamatergic signaling have been mostly focused on NMDA receptor antagonism. Although efficacious in preclinical studies [16,17], NMDA antagonism is limited by severe side effects [18–21], leading to unsuccessful clinical trials [22–25]. One alternative to direct receptor blockade is inhibition of enzymes responsible for glutamate production.

One glutamate-producing enzyme [26,27] upregulated following neuroinflammation as well as other insults [28,29], including those related to aging [30–32] is the glial metallopeptidase glutamate carboxypeptidase II (GCPII), which liberates glutamate from the abundant neuropeptide *N*-acetylaspartyl-glutamate (NAAG). NAAG is the selective endogenous agonist of the metabotropic glutamate receptor mGluR3 located presynaptically and postsynaptically [33]. Presynaptic activation of mGluR3 reduces neurotransmitter release, whereas astrocytic mGluR3 agonism increases glutamate uptake by excitatory amino acid transporters [34,35]. Activation of mGluR3 in the prefrontal cortex of primates positively affects working memory by causing persistent firing on dendritic spines [36–38]. Aging and neuroinflammation can reduce these dendritic spines and impair memory [32,36,39–41].

GCPII inhibitors increase brain NAAG, reduce excitotoxic glutamate release, and provide therapeutic benefit in preclinical models of CNS disease where hyper-glutamatergic transmission is presumed pathogenic including stroke [27], traumatic brain injury (TBI) [42], neuropathy/neuropathic pain [43–45], psychosis [46], addiction [47], and ALS [26,48]. In several of these models, mGluR2/3 antagonist co-administration diminished the GCPII inhibitor effect [42,43,46]. Additionally, GCPII inhibitors improve learning and memory in rodent and nonhuman primate models of schizophrenia [49], stroke/TBI [50], multiple sclerosis (MS) [31,51], and Alzheimer's disease [32,36,40,52]. Memory deficits are observed in mice with the *Rimkla* gene, which encodes NAAG synthetase, knocked out [53]. Taken together, this data suggests a role for NAAG in cognition.

Brain NAAG can be quantified using magnetic resonance spectroscopy (MRS), with 7-Tesla (T) field strengths providing better spectral resolution than lower, more common 1.5 or 3.0 T field strengths [54,55]. Due to spectral overlap between NAA and NAAG peaks, most studies report an additive NAA+NAAG peak of which NAAG accounts for approximately 10% in cortical regions. Additionally, whereas NAAG is an mGluR3 agonist [56], NAA is used as a marker of neuronal health, has roles in neuronal osmoregulation, and is a source of acetate for lipid and myelin synthesis [57]. As NAA and NAAG serve divergent roles, it is critical to investigate them separately. A few studies in disorders other than HIV have selectively quantified brain NAAG and demonstrated significant, positive relationships with cognition. For example, higher NAAG in frontal white matter (FWM) was associated with better visual memory in healthy individuals and in recent onset psychosis [58]. In these same individuals, a missense mutation in the gene, which encodes GCPII, lead to increased GCPII expression, lower NAAG, and related to lower intelligence quotient (IQ) [58]. Higher NAAG in the frontal lobe of individuals with schizophrenia related to better episodic memory [59], whereas higher NAAG in the hippocampus related to better memory and executive function in MS patients [51]. To our knowledge, no previous spectroscopic studies in PWH have examined NAAG.

Considering the overlap between higher order cognitive domains disrupted in older virally suppressed (VS-PWH) [60–62] and domains influenced by NAAG and mGluR3s, we used 7T MRS to investigate relationships between regional NAAG levels and domain-specific cognitive function in VS-PWH. Based on prior studies, we hypothesized that higher NAAG levels in FWM and the hippocampus would relate to better higher order cognition.

Methods

Participants

This analysis included 40 PWH in a MRI/MRS study at the Johns Hopkins Hospital performed from 2013 to 2016 [63,64]. Participants were at least 50 years of age, able to provide written informed consent, and could

ambulate to the clinic. Participants were excluded if they: had a history of opportunistic CNS infection, psychosis, affective, or chronic neurologic disorders; had prior 3-month history of psychoactive drug use; and had contraindications for MR imaging. Exclusion criteria were determined by self-report and medical record review.

Procedures

Following informed consent, participants completed MRI, neurologic, neuropsychological, functional assessments, and a blood draw. Clinical assessments included a neurologic examination and questionnaires assessing demographics, medical, psychiatric, and neurologic history. Reading ability was estimated using the Hopkins Adult Reading Test (HART) [65], depression with the Center for Epidemiological Studies Depression Scale (CES-D) [66], fatigue with the Fatigue Severity Scale [67], sleepiness with the Epworth Sleepiness Scale [68], and HIV dementia with the International HIV Dementia Scale [69]. Functional performance measures included the Karnofsky Performance Scale [70], Columbia Medication Management Scale [71], and the San Diego Finances Test was included as previous studies have shown skills of daily living, such as financial management are correlated with cognitive status [72–74]. Neurocognitive disorder status was determined using the revised American Academy of Neurology practice guideline [75]. $CD4^+$ T-cell counts and HIV RNA levels were quantified. Participants were compensated for participation.

MRI and spectroscopy

The study was performed in a 7.0T scanner (Achieva; Philips Healthcare, Netherlands) using a Nova medical 32-channel receiver coil with a quadrature transmit coil. Details have been previously published [63]. As this is a retrospective analysis of a previously published dataset in older PWH examining a range of spectroscopic metabolites [63], we focused on only NAAG-specific associations here. Based on prior findings in other indications of NAAG-cognition associations, the primary regions of interest (ROI) included left hippocampus, left FWM, and left basal ganglia. Spectra were analyzed in the LCModel software package as described previously [63].

Cognitive function

Given prior studies linking NAAG to higher order cognitive functions, the primary cognitive endpoints included learning and memory, verbal fluency, attention and working memory, and executive function. *Learning and memory* was assessed with the Hopkins Verbal Learning Test Revised (HVLT-R) [76] and Rey–Osterrieth Complex Figure Test [77]; *Verbal fluency* with the Controlled Oral Word Association Test (letters S, N, P) [78] and Animal Fluency; *Attention and WM* with the California Computerized Assessment Package [79]; *executive function* with the interference trial of the Stroop Test and Trail Making Test (TMT)-Part B [80] (for details of measures used, see table, Supplemental Digital Content 1, http://links.lww.com/QAD/D134). To determine the specificity of NAAG-higher order cognition associations, we also examined processing speed, gross, and fine motor function as secondary endpoints. *Processing speed* was measured with TMT-Part A [80] and Digit Symbol [81]; *Gross motor function* with Finger Tapping [80]; and *Fine motor skills* with Grooved Pegboard [80]. All timed measures were log transformed and multiplied by −1 so higher values equated to better performance. Subsequently, within-sample z scores were computed for each outcome so domain-specific outcomes could be combined into composite z scores. The computed cognitive domain z scores were used to examine associations between NAAG and cognition.

Statistical analyses

Extent to which NAAG concentrations predicted domain-specific cognitive function was evaluated using correlations and regressions. First, Pearson's correlations were conducted to evaluate unadjusted relationship between NAAG and cognition. Next, linear regression analyses were conducted when warranted by the correlational analyses to assess the extent to which NAAG concentrations relate to cognition after adjusting for identified depression (depressive symptoms on the CES-D applied as a continuous variable) and reading ability confounders, which were found to be the only significant confounds.

Results

Characteristics of participants

Participants ($N = 40$) were 50–78 years; 55% Black, non-Hispanic and 43% White. All were receiving combination ART **(**Table 1**)**. The most common ART agents included emtricitabine (55%), tenofovir disoproxil fumarate (53%), ritonavir (50%), efavirenz (40%), darunavir (30%), abacavir (22%), and lamivudine (22%). No differences in the relationships between NAAG and cognition were found in participants on efavirenz compared with the rest of the study population. All participants had viral loads (<50 copies/ml), and the average $CD4^+$ count was 696.8 [standard deviation (SD) = 320.7]. The IHDS was used as a screening test and showed a significant proportion of participants were performing at a level of risk for dementia, which is consistent with the fact 75% of participants were found to be impaired in at least two cognitive domains.

Associations between *N*-acetyl-aspartyl glutamate concentrations and cognition

Higher NAAG concentrations in left FWM were associated with better attention/working memory ($r = 0.49$, $P = 0.002$) (Fig. 1). Additionally, higher concentrations in the left hippocampus were associated with better executive

Table 1. Characteristics of the study population and details of cognitive battery.

	VS-PWH ($n = 40$)
Demographics	
Age [M (SD)]	59.0 (5.6)
Male [*n* (%)]	29 (73)
Years of education [M (SD)]	14.6 (2.8)
HART-estimated IQ score [M (SD)]	108.8 (13.5)
Race [*n* (%)]	
White	17 (43)
Black	22 (55)
Asian	1 (2)
Clinical and functional assessments [M (SD)]	
CES-D total score (range 0-60; higher more symptoms)	6.0 (13)
Fatigue Severity Scale (range 0–7; higher more symptoms)	3.88 (1.5)
Epworth Sleepiness Scale (range 0–24; higher more symptoms)	9.5 (5.1)
International HIV Dementia Scale (range 0–12; higher better)	10.2 (1.6)
Cognitive impairment on ≥2 domains[a]	30 (75)
Columbia Medication Management Scale (range 0–16; higher better)	11.6 (4.2)
San Diego Finances Test (range 0–22; higher better)	20.6 (1.6)
Karnofsky Performance Scale (range 0–100; higher better)	88.0 (9.7)
NAAG [M (SD)]	
Left frontal white matter	1.26 (0.31)
Left basal ganglia	1.35 (0.99)
Left hippocampus	1.59 (0.83)
Mesial precuneus	0.67 (0.32)
Mesial posterior cingulate cortex	0.85 (0.23)
NP test performance by domain[c] [M (SD)]	
Learning and memory	0.00 (0.77)
Verbal fluency	0.05 (0.87)
Attention/working memory	0.08 (0.78)
Executive function	−0.02 (0.39)
Processing speed	0.00 (0.88)
Gross motor function	0.05 (0.90)
Fine motor skills	−0.02 (0.94)

M, mean; NP, neuropsychological; PWH, people with HIV; SD, standard deviation; VS, virally suppressed.
[a]Cognitive impairment based on the revised American Academy of Neurology 'Frascati' criteria.
[b]Log transformed outcomes.
[c]Composite *z* score.

function ($r = 0.39$, $P = 0.04$) whereas higher left basal ganglia NAAG was associated with better fluency ($r = 0.36$, $P = 0.03$). In adjusted analyses, the positive association between left FWM NAAG and attention/white matter remained significant ($\beta = 0.41$, $P = 0.01$) as did the positive association between left basal ganglia NAAG and fluency ($\beta = 0.26$, $P = 0.04$). The relationship between left hippocampus NAAG and ejection fraction was minorly altered but no longer met significance ($\beta = 0.39$, $P = 0.05$).

To determine the specificity of these findings to higher order cognitive domains, NAAG concentrations in the primary ROI were examined in relation to lower order domains such as processing speed, fine, and gross motor function (see heat map, Supplemental Digital Content 2, http://links.lww.com/QAD/D135). None of the associations with these more basic measures met statistical significance. To determine the specificity of these findings to specific brain regions hypothesized to be involved with the higher order domains of interest, associations were examined between NAAG in the mesial precuneus and mesial posterior cingulate cortex (PCC) and the primary cognitive endpoints. Interestingly, higher concentrations in the mesial precuneus were associated with poorer attention/working memory ($r = -0.44$, $P = 0.01$) and this association remained after adjusting for confounders ($\beta = -0.39$, $P = 0.03$).

Discussion

MRS is an increasingly utilized tool for identifying neurometabolite differences in a range of conditions, including HIV. Previously, PWH were shown to have higher myoinositol and choline (markers of inflammation) and lower levels of NAA (a signal of neuronal loss or dysfunction) [82,83]. Lower levels of glutamate in PWH have also been associated with cognitive impairment [84,85]. However, to our knowledge, this is the first study examining the NAAG-cognition associations in PWH. NAAG has been understudied in prior spectroscopy studies, mainly because of technical difficulties in accurately extracting NAAG from the larger NAA peak [86]. The use of a 7T field-strength magnet for collection of the presented data allowed accurate NAAG measurements.

We report significant, positive associations between regional NAAG and cognition. FWM contains the

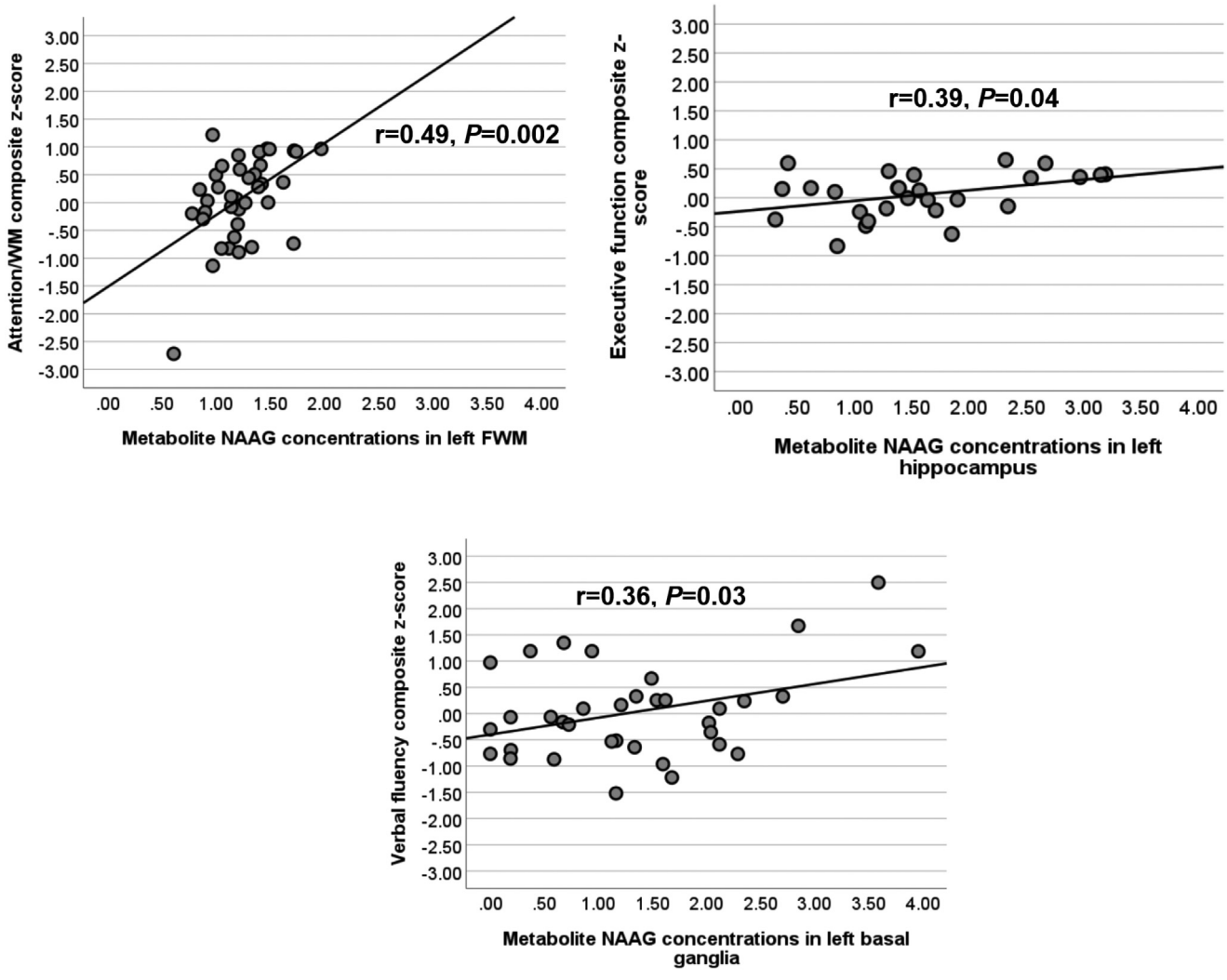

Fig. 1. Significant associations between brain *N*-acetyl-aspartyl glutamate and cognition in virally suppressed people with HIV. Regional NAAG levels are differentially associated with cognitive domain scores. MRS NAAG levels in left FWM are positively associated with the attention/WM cognitive domain score. NAAG levels in the Hippocampus are associated with executive function, and left basal ganglia NAAG is positively correlated with verbal fluency. FWM, frontal white matter; NAAG, *N*-acetyl-aspartyl glutamate; *r*, Pearson correlation coefficient; WM, working memory.

highest level of NAAG, which allows for accurate spectral estimations [87]. We found left FWM NAAG positively correlated with attention/working memory, a relationship previously seen in healthy individuals and in people with recent onset psychosis [58]. NAAG levels in the left hippocampus were positively associated with executive function, which has also been observed in MS [51]. One novel finding here was the positive correlation between left basal ganglia NAAG and verbal fluency. This was not unexpected as, similar to the hippocampus, the left basal ganglia is modulated by mGluR3, where NAAG is the endogenous agonist [88–90]. In addition, the size and neurotransmitter balance in the left basal ganglia are disrupted in HIV [91]. The connection between left basal ganglia and fluency aligns with prior data in PWH, which identified a link between left basal ganglia size and performance on similar word association tests [92].

The overlap between results observed in the current study, recent onset psychosis, and MS suggest this pro-cognitive relationship may be broadly applicable across disorders [51,58]. Interestingly, NAAG-cognitive domain relationships are region-specific. Secondary analyses revealed a previously undescribed negative relationship between mesial precuneus NAAG and attention/working memory. As NAAG is thought to contribute to complex cognitive tasks (e.g. working memory, executive function), it is plausible it plays a different role in brain regions deactivated by cognitive tasks. Additional studies are needed to further elucidate these findings. As mentioned in the introduction, cART therapy has significantly prolonged the lives of PWH, which means a larger proportion of the population is reaching older ages and are at risk of developing worsening cognitive deficits. There is a clinically unmet need to develop novel

therapeutics to address cognitive changes in PWH. Glutamatergic excitotoxicity is hypothesized to be one mechanism through which HIV-associated cognitive changes can occur. Inhibition of GCPII (the enzyme that breaks down NAAG) has been shown preclinically to mitigate excitotoxicity [28,42], additionally co-administration of a GCPII inhibitor with a glutaminase inhibitor in a cancer model has been shown to alter glutamate release even more extensively [93]. NAAG has been found co-localized with a range of neurotransmitters including glutamate, dopamine, serotonin, GABA, and choline [94,95], leading to the hypothesis that NAAG is able to modulate release of multiple neurotransmitters. As GCPII inhibition has preclinical benefit in schizophrenia and MS models [31,49] where positive NAAG/cognition associations are seen in patients, it is worth investigating the therapeutic benefit of GCPII inhibition in preclinical HIV models.

There are study limitations, which should be noted. As the analyses done for this study were exploratory to identify potential relationships between NAAG and cognition, values were not corrected for multiple comparisons. Future studies will need to be adequately powered to allow for correction for multiple comparisons to validate the reported observations. We used a within-sample z-score approach to generate cognitive domain scores, so the z scores in the domains are not reflective of cognitive performance compared with published norms. PWH were on older ART regimens as the data was collected between 2013 and 2016, so the data may not be wholly representative of people on current combination regiments, which often include highly effective integrase inhibitors [96,97]. Additionally, nearly half of participants were taking Efavirenz, which has neurotoxic effects and is no longer routinely used [98], though our analyses did not show Efavirenz as a confound. The largely male study population were over the age of 50 years, and already at increased risk for cognitive impairment. Participants were excluded if diagnosed with affective disorders, which may limit generalizability as these conditions are common in PWH. As this was a retrospective analysis, we also did not have nadir $CD4^+$ count, viral load in cerebrospinal fluid, or measures of neuroinflammation. Future directions include a longitudinal study to investigate if NAAG levels can be predictive of long-term cognitive function across a wider age-range, across genders, and in context of current ART regimens (e.g. integrase inhibitors). These longitudinal studies should also include intra-patient assessment of ART treatment history as well as blood and CSF markers of inflammation.

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Conflicts of interest

There are no conflicts of interest.

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