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# A candidate gene study of intermediate histopathological phenotypes in HIV-associated neurocognitive disorders

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## Abstract

HIV-associated neurocognitive disorders (HAND) describe a spectrum of neuropsychological impairment caused by HIV-1 infection. While the sequence of cellular and physiological events that lead to HAND remains obscure, it likely involves chronic neuroinflammation. Host genetic markers that increase the risk for HAND have been reported, but replication of such studies is lacking, possibly due to inconsistent application of a behavioral phenotype across studies. In the current study, we used histopathologic phenotypes in order to validate putative risk alleles for HAND. The National NeuroAIDS Tissue Consortium, a longitudinal study of the neurologic manifestations of HIV. Data and specimens were obtained from 175 HIV-infected adults. After determining several potential covariates of neurocognitive functioning, we quantified levels of six histopathological markers in the frontal lobe in association with neurocognitive functioning: SYP, MAP 2, HLA-DR, Iba1, GFAP, and  $\beta$ -amyloid. We then determined alleles of 15 candidate genes for their associations with neurocognitive functioning and histopathological markers. Finally, we identified the most plausible causal pathway based on our data using a multi-stage linear regression-based mediation analysis approach. None of the genetic markers were associated with neurocognitive functioning. Of the histopathological markers, only MAP 2 and SYP were associated with neurocognitive functioning; however, MAP 2 and SYP did not vary as a function of genotype. Mediation analysis suggests a causal pathway in which presynaptic degeneration (SYP) leads to somatodendritic degeneration (MAP 2) and ultimately neurocognitive impairment. This study did not support the role of host genotype in the histopathology underlying HAND. The findings lend further support for synaptodendritic degeneration as the proximal underlying neuropathological substrate of HAND.

**Keywords** HIV-associated neurocognitive disorder · Genetic · Histopathology · Neuropathology · NeuroAIDS · Synaptodendritic

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## Introduction

Pharmaceutical advances stemming from immunologic and genetic research have greatly improved and extended the lives of people living with HIV-1 (PLWH). Despite this, the prevalence of neurocognitive deficits in PLWH, collectively termed HIV-associated neurocognitive disorders (HAND), remains high (Antinori et al., 2007; Heaton et al., 2011). Prior to the widespread use of combination antiretroviral therapy (cART), neurocognitive syndromes due to HIV-1 infection were often severe and largely considered the manifestation of HIV encephalitis (McArthur et al., 1993; Moore et al., 2006; Glass et al., 1995; Bell et al., 1998; Persidsky & Gendelman, 2003; Everall et al., 2005; Letendre et al., 2011; Boven et al., 2000; Conant et al., 1998; Eugenin et al., 2006; Kraft-Terry et al., 2009). However, HAND currently presents with milder symptoms in the vast majority of cART-treated cases (McArthur et al., 2005; Heaton et al., n.d.) and is not typically associated with HIV encephalitis (Everall et al., 2009). Instead, the neuropathogenesis of HAND is now believed to be largely the result of neurodegeneration driven by chronic neuroinflammation (Glass et al., 1995; Persidsky & Gendelman, 2003; Kraft-Terry et al., 2009; Everall et al., 2009).

Candidate gene studies have identified functional variants, largely within immune-related genes, that modify risk for HAND (as reviewed in (Levine et al., 2014a; Kallianpur & Levine, 2014)). Such findings make sense biologically, supporting the role of inflammation in HAND pathogenesis. For example, some chemokines affect neuronal signaling with subsequent disturbance of glial and neuronal functions (Zheng et al., 2001), while others serve to block the HIV-1 co-receptor, thus mitigating HIV-1 replication (Lane et al., 2003) and slowing disease progression (Gonzalez et al., 2001; Gonzalez et al., 2005), as well as reducing macrophage activation and chemotaxis of monocytes and other cells into the brain (Kaul & Lipton, 2005; Weiss et al., 1999). In addition to immune-related genes, candidate gene studies have implicated dopaminergic dysregulation (Levine et al., 2014a; Kumar et al., 2009; Kumar et al., 2011; Levine et al., 2012; Levine et al., 2014b), variation in mitochondrial function (Samuels et al., 2016; Hulgán et al., 2015), and cellular lipid and cholesterol transport in HAND pathogenesis, as recently reviewed in (Geffin & McCarthy, 2018). However, very few candidate gene markers have been replicated in subsequent studies by independent groups. One likely reason for this is that the vast majority of these studies utilized behavioral phenotypes (e.g., HAND diagnosis or neurocognitive functioning), with little consistency between studies. Due to the inherent limitations of neurocognitive assessment (e.g., measurement error due to tests' psychometric properties and engagement by the examinee), coupled with the poor inter-rater agreement of what distinguishes HAND from other causes of

neurocognitive impairment (Woods et al., 2004), a more fruitful strategy may be to focus on histopathological phenotypes.

Putative immunohistochemical markers of the neuropathological changes underlying HAND include synaptophysin (SYP) and microtubule-associated protein-2 (MAP 2) (Moore et al., 2006), abnormal protein aggregates such as  $\beta$ -amyloid (Achim et al., 2009; Rempel & Pulliam, 2005; Green et al., 2005; Esiri et al., 1998; Soontornniyomkij et al., 2012), and markers of microglial/macrophage activation, astroglial activation, and dysregulated cytokine expression (Glass et al., 1995; Bell et al., 1998; Persidsky & Gendelman, 2003; Everall et al., 2005; Letendre et al., 2011; Boven et al., 2000; Conant et al., 1998; Eugenin et al., 2006; Kraft-Terry et al., 2009). If previously identified genetic variants modify risk for HAND, it logically follows that those variants also modify the cellular and pathophysiological pathways that underlie HAND. Bridging the informational gap between genotypes and behavioral phenotypes in the context of HAND may provide important insights about pathogenesis. We recently reported results of an ambitious study that bridged genetic, histopathological, virologic, and neurocognitive data within subjects in order to understand which histopathological features and genetic variants were relevant to HAND pathogenesis (Levine et al., 2015). That study identified several genetic susceptibility loci that influenced histopathology and other disease parameters. Most notably, neurocognitive functioning was strongly correlated with levels of MAP 2 and SYP in the frontal cortex, both of which declined as plasma HIV-1 RNA viral load increased. This underscores the widely reported process by which HIV-1 replication-related events lead to synaptodendritic degeneration. Furthermore, an inverse relationship between SYP expression and  $\beta$ -amyloid plaque burden in frontal cortex suggests that HIV-1 replication in the brain may be a driver of the histopathological changes or, alternatively, an initiator of a causal chain of events involving neuroinflammation (reflected by ionized calcium-binding adapter molecule-1 (Iba1)) and dysfunctional protein clearance (reflected by  $\beta$ -amyloid plaque deposition). Downstream to these changes is synaptodendritic degeneration which is the immediate histopathological substrate of HAND, although several factors likely modify this cascade (Guha et al., 2018; Cantres-Rosario et al., 2019; Santerre et al., 2019; Desplats et al., 2013) and the ultimate manifestation of HAND (Geffin & McCarthy, 2018; Saloner et al., 2019; Yu et al., 2019; Tovar-y-Romo et al., 2012; Gannon et al., 2017).

In the current study, we expanded upon the previous findings (Levine et al., 2015). In the previous paper, we were unable to examine how demographic factors, HIV-1 disease variables, and host genotypes predicted MAP 2 and SYP levels due to the low number of cases. In the current study, we first revisited this relationship by examining data from a larger sample of PLWH. Second, we assessed whether a

variety of histopathological markers were associated with neurocognitive functioning among the larger sample. Third, we genotyped several additional genetic susceptibility loci and tested for association with the histopathological markers within the frontal cortex. Finally, we examined the causal pathway between these histopathological markers and global neuropsychological functioning. Our goal was to exploit knowledge of functional polymorphisms to identify the relevant genes and histopathological markers involved in HAND pathogenesis.

## Methods

Biological specimens were obtained from the National NeuroAIDS Tissue Consortium (NNTC) (Morgello et al., 2001). The NNTC is a longitudinal study of neuroAIDS in existence since 1998. Specimens and data used in the current study generally came from participants recruited because they had one or more diagnoses indicative of advanced HIV-1 disease, were at high risk of death, and agreed to participate in the study evaluations and to donate their organs for research purposes. Participants were typically evaluated every 6–12 months, undergoing comprehensive neurocognitive testing, psychiatric/substance use interview, and neuromedical evaluation. Upon death, brains were harvested for research purposes. Demographic and HIV-1 disease characteristics of individuals included in the current study are shown in Table 1.

For inclusion, all cases were required to be HIV-seropositive, 18 years or older, and diagnosed as either neurocognitively normal or with HAND within 1 year prior to death, per established research criteria (Antinori et al., 2007; No authors, 1996). Those determined to be neurocognitively impaired due to other causes were not included. All cases died well into the cART era (post-1996). Exclusion criteria were (1) pre- or postmortem evidence of non-HIV-related neurological diseases (e.g., stroke, neoplasm, multiple sclerosis, traumatic brain injury, and neurodegenerative illness) and (2) history or evidence of central nervous system (CNS) toxoplasmosis or progressive multifocal leukoencephalopathy. Comorbid medical conditions were in most cases self-reported by participants during the visits just prior to death, and were confirmed via chart review whenever possible. Sample characteristics are displayed in Table 1.

### DNA extraction and genotyping

DNA extraction and genotyping methods are provided in the [Supplemental Material](#). Table 2 displays the genes, specific reference SNP cluster ID numbers, and gene function.

## Clinical variables

### Neurocognitive functioning

Neuropsychological clinical ratings were determined for each case based on neurocognitive test scores obtained within 1 year of death. A global ability rating was derived from demographically corrected *T*-scores from a comprehensive neuropsychological battery, as previously described (Woods et al., 2004). Clinical ratings were assigned on a scale that ranged from 1 (above average) to 9 (severely impaired), with scores greater than or equal to 5 indicative of at least mild impairment. These were summarized as a global clinical rating (GCR). Among PLWH, the GCR was associated with activities of daily living (Heaton et al., 2004), HIV-1 disease variables (Heaton et al., 2011), and synaptodendritic changes on brain histopathology (Moore et al., 2006).

### HIV-1 disease measures

Peripheral blood was collected from living participants by venipuncture into EDTA and heparinized tubes prior to death and was assayed using the Roche Amplicor Assay for HIV-1 RNA viral load and by flow cytometry for CD4+ T lymphocyte subsets. Plasma HIV-1 RNA viral load was measured at the last premortem visit within 1 year of death. Plasma measures of viral load and CD4+ T cell count were not available at the time of death because venipuncture cannot be performed after the heart has ceased beating due to intravascular blood coagulation. Duration of HIV-1 infection was based on self-reported date of infection and confirmed by chart review when possible.

### Antiretroviral CNS penetration or effectiveness

We employed the antiretroviral CNS penetration or effectiveness (CPE), a score that is based on the pharmacologic characteristics of antiretroviral medications (Letendre, 2011). The CPE of individual antiretroviral drugs is ranked from 1 (poorest) to 4 (best) based on the 2010 ranking system (Letendre et al., 2010). The CPE score for each case was derived by adding ranks of all antiretroviral drugs within the regimen, which was reported at the time of neurocognitive testing. Higher scores indicated a regimen with increased penetration of the blood-brain barrier.

### Alcohol and substance use

The Psychiatric Research Interview for Substance and Mental Disorders (PRISM) (Hasin et al., 1996) or Composite International Diagnostic Interview (CIDI) (Robins et al., 1988) was used to ascertain lifetime substance use disorders. Both are structured diagnostic interviews that yield DSM-IV

**Table 1** Sample characteristics

Age at death	47.4 years <sup>a</sup> (SD = 9.2)
Length of HIV infection	12 years (SD = 6.4)
CD4+ T cell count	122 (SD = 168)
Median plasma HIV-1 RNA viral load	15,406 copies/mL
CPE	8.96 (SD = 4.08)
Global clinical rating	5.31 (SD = 1.87)
	Percent <sup>b</sup> of sample ( <i>n</i> )
Detectable (> 50 copies/mL) plasma HIV-1 RNA viral load	84% (Hulgan et al., 2015)
Male	81.4% (162)
Race/ethnicity	
Caucasian	50.8% (100)
African American	24.9% (No authors, 1996)
Hispanic	22.3% (Saloner et al., 2019)
Asian/Native Alaskan	2% (Moore et al., 2006)
Major depression	
Current	23.4% (Woods et al., 2004)
Past	16.8% (Kaul & Lipton, 2005)
Substance use disorder	
Current	20.3% (Geffin & McCarthy, 2018)
Past	29.4% (Yu et al., 2019)
Alcohol use disorder	
Current	9% (McArthur et al., 2005)
Past	26% (Guha et al., 2018)
HIV encephalitis	9.2% (Everall et al., 2009)
HAND	74.2% (135)
ANI	14.2% (Kumar et al., 2011)
MND	31% (Benjamini & Hochberg, 1995)
HAD	29% (Hasin et al., 1996)

*SD* standard deviation, *CPE* central nervous system penetration or effectiveness, *ANI* asymptomatic neurocognitive impairment, *MND* mild neurocognitive disorder, *HAD* HIV-associated dementia

<sup>a</sup> Values presented as means unless otherwise indicated

<sup>b</sup> Percentages are the proportion of those with available data

diagnoses. For the purposes of the current study, NNTC participants were classified with none, current, or past substance use disorder for the following drugs: cocaine, opioids, and methamphetamine. Alcohol use was similarly classified.

### Immunohistochemistry and histopathological characterization

As described in (Moore et al., 2006; Masliah et al., 1997), brain tissue was harvested from deceased HIV-seropositive NNTC participants as soon as possible after death. Tissue blocks measuring 4 cm<sup>3</sup> were taken from the right dorsolateral midfrontal cortex. The blocks were fixed overnight in 4% paraformaldehyde and cut at 40 μm thick with a Leica Vibratome (Vienna, Austria). Histopathological characterization was accomplished using previously described

methods (Moore et al., 2006; Masliah et al., 1997) based on immunohistochemistry conducted on formalin-fixed paraffin-embedded sections. The midfrontal gyrus was processed for the following markers: SYP (gray matter), MAP 2 (gray matter), human leukocyte antigen-DR (HLA-DR, gray and white matter separately), Iba1 (gray and white matter separately), glial fibrillary acidic protein (GFAP, gray and white matter separately), and β-amyloid (gray matter). Additional details of immunohistochemistry analysis and quantitative image analysis are in the [Supplemental Material](#).

### Statistical analysis

Significance was assessed at a false discovery rate (FDR) of 0.05 (Benjamini & Hochberg, 1995).

**Table 2** Candidate genes and their protein's primary function

Gene	Full name	Reference SNP cluster ID	Protein function
<i>IL6</i>	Interleukin 6	rs1800796	Pro-inflammatory cytokine
<i>CCL3</i>	C-C motif chemokine ligand 3	rs1719134	Chemokine involved in recruitment and activation of granulocytes
<i>HCP5</i>	HLA complex P5	rs2395029	A human endogenous retrovirus. Variants confer protection against AIDS
<i>CX3CR1</i>	C-X3-C motif chemokine receptor 1	rs3732379	Chemokine involved in adhesion and migration of leukocytes
<i>HFE</i>	Homeostatic iron regulator	rs1799945	Regulates circulating iron uptake via regulating interaction between transferrin receptor and transferrin
<i>NFE2L2</i>	Nuclear factor, erythroid 2 like 2	rs6706649	Transcription factor that regulates expression of antioxidant proteins in response to injury and inflammation
<i>CXCL12</i>	C-X-C motif chemokine ligand 12	rs1801157	Chemokine that activates leukocyte migration in CNS as part of inflammatory activation
<i>CCR2</i>	C-C motif chemokine receptor 2	rs1799864	Chemokine that mediates monocyte chemotaxis
<i>BDNF</i>	Brain-derived neurotrophic factor	rs6265	Supports cell survival in CNS
<i>HEPH</i>	Hephaestin	rs1264212	Involved in metabolism and homeostasis of iron
<i>TNF</i>	Tumor necrosis factor	rs1800629	Pro-inflammatory cytokine
<i>CD33</i>	CD33 molecule (SIGLEC3)	rs3865444	Involved in inhibition of phagocytosis within cells
<i>CCL2</i>	C-C motif chemokine ligand 2	rs1024611	Pro-inflammatory cytokine
<i>MBL2</i>	Mannose-binding lectin 2	rs1800450 rs1800451 rs5030737	Involved in innate immune response
<i>APOE</i>	Apolipoprotein E	rs429358 rs7412	Involved in lipid transport and metabolism

SNP single nucleotide polymorphism, CNS central nervous system

All genetic loci except *MBL2* and *APOE* were treated as having a dominant acting risk allele. *MBL2* genotypes were treated as three categories (A/A, A/O, and O/O). To test the association of *APOE*  $\epsilon 4$  and  $\epsilon 2$  alleles with GCR, we modeled dominant main effects and an interaction between  $\epsilon 4$  and  $\epsilon 2$  in order to allow the inclusion of participants carrying the *APOE*  $\epsilon 2/\epsilon 4$  genotype. Symbolically, this model was:

$$GCR_i = \alpha + \beta_{\epsilon 4} I_{\{i, \epsilon 4/\epsilon 4, \epsilon 4/\epsilon 3, \epsilon 4/\epsilon 2\}} + \beta_{\epsilon 2} I_{\{i, \epsilon 2/\epsilon 2, \epsilon 2/\epsilon 3, \epsilon 4/\epsilon 2\}} + \gamma \epsilon 4 \epsilon 2 I_{\{i, \epsilon 4/\epsilon 4, \epsilon 4/\epsilon 3, \epsilon 4/\epsilon 2\}} I_{\{i, \epsilon 2/\epsilon 2, \epsilon 2/\epsilon 3, \epsilon 4/\epsilon 2\}} + e_i$$

where  $I_{\{\epsilon 4/\epsilon 4, \epsilon 4/\epsilon 3, \epsilon 4/\epsilon 2\}} = 1$  if individual  $i$ 's genotype was  $\epsilon 4/\epsilon 4$ ,  $\epsilon 4/\epsilon 3$ , or  $\epsilon 4/\epsilon 2$  and  $= 0$  otherwise,  $I_{\{\epsilon 2/\epsilon 2, \epsilon 2/\epsilon 3, \epsilon 4/\epsilon 2\}} = 1$  if individual  $i$ 's genotype was  $\epsilon 2/\epsilon 2$ ,  $\epsilon 2/\epsilon 3$ , or  $\epsilon 4/\epsilon 2$  and  $= 0$  otherwise, and  $e_i$  was the residual error. If the evidence for interaction was not significant, we reduced the model to:  $GCR_i = \alpha + \beta_{\epsilon 4} I_{\{i, \epsilon 4/\epsilon 4, \epsilon 4/\epsilon 3, \epsilon 4/\epsilon 2\}} + \beta_{\epsilon 2} I_{\{i, \epsilon 2/\epsilon 2, \epsilon 2/\epsilon 3, \epsilon 4/\epsilon 2\}} + e_i$

To test potential causal pathways involving histopathological markers, we used a multi-stage linear regression-based mediation analysis approach (MacKinnon, 2008; Tingley et al., 2014). The evidence for a particular pathway was provided by the proportion of the effect of a predictor that could be explained by the effect of a mediator (the proportion mediated). The proportion mediated was determined by conducting two linear regressions: (1) regressing the mediator

on the predictor and (2) regressing the outcome on the mediator and the predictor. Additional covariates could be included in both regressions if desired.

## Results

We first determined if potential confounders were associated with GCR (Table 3), including age at death, sex (female or reference group male), duration of HIV-1 infection, log<sub>10</sub> plasma HIV-1 RNA viral load (herein referred to as viral load), NNTC site (reference group site 1), CD4+ T cell count, CPE score, self-reported race/ethnicity (African American, Hispanic, Asian/Native Alaskan, or reference group Caucasian), alcohol use (current, past, or reference group none), or drug use (current, past, or reference group none). Only viral load was associated with GCR using a significance threshold of 0.05. Because the inclusion of race/ethnicity could reduce the effects of population stratification that

confounded genetic association analyses, we included race/ethnicity in our subsequent analyses despite lack of statistical significance.

We next tested each of the histopathological markers' association with GCR, controlling for the effects of race/ethnicity and viral load (Table 4). Only MAP 2 and SYP were associated with GCR at an FDR of 0.05. We then determined whether the potential confounders were associated with MAP 2 or SYP. The viral load was significantly associated with SYP. None of the potential confounder variables were associated with MAP 2 (Table 3).

### Association of Genotype with GCR, SYP, and MAP 2

We next determined candidate genetic markers' associations with GCR, SYP, or MAP 2. Because genetic associations could be confounded by population stratification, we included race/ethnicity as a covariate in all subsequent analyses despite its lack of statistical significance. When using a nominal per

**Table 3** Potential confounding covariates of GCR, SYP, and MAP 2

Covariate	GCR			SYP			MAP 2		
	Estimate	<i>p</i> value	<i>n</i>	Estimate	<i>p</i> value	<i>n</i>	Estimate	<i>p</i> value	<i>n</i>
Age at death	-0.022	0.156	175	0.005	0.915	102	0.007	0.875	102
Sex: Female	-0.429	0.227	175	-0.905	0.404	102	-0.316	0.763	102
NNTC site <sup>b</sup>									
Site 2	0.596	0.148 <sup>a</sup>	175	NA	NA	NA	NA	NA	NA
Site 3	0.890			-0.123	0.880	102	-0.244	0.757	102
Site 4	0.427			NA	NA	NA	NA	NA	NA
Duration of HIV infection	-0.043	0.051	170	-0.006	0.919	98	-0.017	0.775	98
Log <sub>10</sub> plasma HIV-1 RNA viral load	0.272	<i>0.004</i>	169	-0.600	<i>0.040</i>	95	-0.442	0.119	95
CD4+ T cell count	-0.002	0.069	169	0.004	0.104	97	0.003	0.240	97
CPE	0.061	0.131	145	0.011	0.906	83	0.118	0.194	83
Race/ethnicity <sup>c</sup>									
African American	-0.498	0.148 <sup>a</sup>	173	1.331	0.201	100	0.607	0.543	100
Hispanic	0.235	-	-	0.101	-	-	0.296	-	-
Asian/Native Alaskan	0.969	-	173	-1.074	-	-	0.957	-	-
Alcohol use									
Current	0.58	0.271 <sup>a</sup>	151	2.71	0.133 <sup>a</sup>	71	0.155	0.930 <sup>a</sup>	71
Past	0.416	-	-	-1.376	-	-	-1.023	-	-
Drug use									
Current	0.273	0.493 <sup>a</sup>	150	0.239	0.870 <sup>a</sup>	70	-0.528	0.704 <sup>a</sup>	70
Past	0.462	-	-	-0.507	-	-	-0.574	-	-

Statistically significant values are shown in italic

GCR global clinical rating, SYP synaptophysin, MAP 2 microtubule-associated protein-2, NNTC National NeuroAIDS Tissue Consortium, CPE central nervous system penetration or effectiveness, NA not applicable

<sup>a</sup>Omnibus *p* value

<sup>b</sup>Site 1 as the reference site

<sup>c</sup>Caucasian as the reference group

**Table 4** Histopathological marker associations with GCR controlling for race/ethnicity and plasma HIV-1 RNA viral load

	Effect size	<i>p</i> value <sup>a</sup>	<i>n</i>
Iba1 gray matter	-0.0176	0.2259	161
Iba1 white matter	-0.0234	0.0499	161
HLA-DR gray matter	0.0227	0.7310	160
HLA-DR white matter	0.0224	0.4039	160
GFAP gray matter	0.3357	0.1133	161
GFAP white matter	-0.1112	0.5873	161
β-amyloid gray matter	0.3496	0.1299	161
MAP 2 gray matter	-0.1889	0.0003	82
SYP gray matter	-0.1518	0.0031	82

*Iba1* ionized calcium-binding adapter molecule-1, *HLA-DR* human leukocyte antigen-DR, *GFAP* glial fibrillary acidic protein, *MAP 2* microtubule-associated protein-2, *SYP* synaptophysin

<sup>a</sup> *p* values shown are not corrected for false discovery rate (FDR)

test *p* value cutoff of 0.05, *HFE*, *HEPH*, and *MBL2* were associated with GCR, *NFE2L2*, and *CCR2* were associated with SYP, and *NFE2L2*, and *HCP5* were associated with MAP 2. However, none of the genetic markers were significantly associated with GCR, SYP, or MAP 2 at an FDR of 0.05 (Table 5). Thus, when further testing for the association of MAP 2 and SYP with GCR, we did not include genetic markers.

### Mediation analysis

When both MAP 2 and SYP were simultaneously included as predictors, MAP 2, but not SYP, was associated with GCR (Table 6). This suggests the relationship of SYP and GCR is mediated by MAP 2; however, there was strong collinearity between MAP 2 and SYP. To test this potential pathway, we used mediation analyses to determine the most plausible pathway that could explain the observed association of SYP, MAP 2, and GCR. We assumed that GCR would not influence SYP or MAP 2; therefore, we limited our analyses to four possible pathways: (1) the effect of SYP on GCR was mediated through MAP 2 (SYP → MAP 2 → GCR); (2) the effect of MAP 2 on GCR was mediated through SYP (MAP 2 → SYP → GCR); (3) neither SYP nor MAP 2 was a mediator of the other; and 4) a more complex pathway in which both MAP 2 and SYP mediated the effects of each other, possibly due to unmeasured variables. If pathway 1 (SYP → MAP 2 → GCR) was the predominant pathway, we would expect the mediation effects to be significant for this pathway and not for pathway 2 (MAP 2 → SYP → GCR). If pathway 2 was the predominant pathway, the mediation effects would be significant for this pathway and not for pathway 1. If neither SYP nor MAP 2 mediated, neither analysis

would be statistically significant. If both SYP and MAP 2 had direct and mediated effects, both analyses would be expected to be statistically significant. Based on the mediation analyses, the statistical support for pathway 1 (Fig. 1) was much stronger than that for pathway 2 (Fig. 2). Therefore, these results were most consistent with the SYP → MAP 2 → GCR causal pathway.

### Discussion

In the current study, we took several steps to address the question of whether or not host genotype affected the histopathological factors that underlay the neurocognitive dysfunction common to PLWH. The first step involved determining if the quantitative measure of neurocognitive functioning (i.e., GCR) was associated with virologic, demographic, mood, CNS penetration of antiretroviral medication, and/or substance use factors. In this sample, only plasma HIV-1 RNA viral load was significantly associated with GCR. This finding might be somewhat unexpected considering that recent studies did not find viral load to be associated with cognitive functioning, at least in the current era of widespread cART use (Clifford & Ances, 2013). However, the NNTC cohort is composed of individuals with more advanced illness, in which viral load was likely higher and cognitive functioning poorer than in clinical cohorts of living PLWH in general. Furthermore, the higher viral load might reflect poor medication adherence and health in general, which suggests that other medical factors not captured in the current analysis (e.g., metabolic syndrome and comorbid medical illnesses) may be contributing. The lack of association between demographics and GCR may be due to the use of normative data when interpreting neurocognitive test scores. That is, standardized scores were derived using age, education, gender, and/or ethnicity stratified normative data. Neither current nor past substance or alcohol use disorder was associated with GCR, despite our focus on the most neurotoxic drugs (cocaine, methamphetamine, and opioids). This is somewhat surprising considering that the majority of past studies have reported an increased risk of neurocognitive impairment associated with substance use (Martin et al., 2018; Thaler et al., 2015; Persidsky et al., 2011; Meyer et al., 2013). One possible explanation is that the relatively advanced disease stage of our sample, with concomitant higher rates of severe cognitive impairment (i.e., HIV-associated dementia), might simply overshadow the damaging effects of alcohol or substance use. Finally, as a group, our sample was neurocognitively impaired based on the average GCR. The reason for this impairment may not have been captured by our study due to incomplete data collection, as mentioned above. Among those important variables not captured were comorbid medical conditions, which in more recent studies appeared to be among the



**Table 5** Association of genetic loci with GCR, SYP, and MAP 2

Genotype <sup>a</sup>	GCR			SYP			MAP 2		
	Effect size	<i>p</i> value <sup>b</sup>	<i>n</i>	Effect size	<i>p</i> value <sup>b</sup>	<i>n</i>	Effect size	<i>p</i> value <sup>b</sup>	<i>n</i>
<i>IL6</i>	-0.43	0.303	148	1.122	0.346	88	-0.075	0.947	88
<i>CCL3</i>	-0.250	0.454	145	0.568	0.561	86	-0.116	0.901	86
<i>HCP5</i>	-0.214	0.800	148	-1.621	0.517	88	-5.451	0.018	88
<i>CX3CR1</i>	-0.357	0.275	148	0.562	0.571	88	0.873	0.354	88
<i>HFE</i>	-1.019	0.004	148	0.766	0.517	88	0.273	0.809	88
<i>NFE2L2</i>	0.091	0.858	148	3.402	0.017	88	2.914	0.032	88
<i>CXCL12</i>	0.129	0.721	148	-1.148	0.253	88	-0.721	0.453	88
<i>CCR2</i>	0.161	0.652	148	2.678	0.007	88	1.749	0.070	88
<i>BDNF</i>	-0.319	0.365	148	1.023	0.306	88	-0.442	0.644	88
<i>HEPH</i>	0.670	0.037	148	-1.601	0.084	88	-0.754	0.399	88
<i>TNF</i>	0.483	0.198	146	0.895	0.428	88	1.289	0.228	88
<i>CD33</i>	-0.617	0.056	147	1.163	0.263	87	1.255	0.215	87
<i>CCL2</i>	-0.508	0.102	148	0.600	0.516	88	0.356	0.686	88
<i>MBL2</i>			148			88			88
A/O	0.422	0.027 <sup>c</sup>		1.025	0.118 <sup>c</sup>		-0.239	0.310 <sup>c</sup>	
O/O	2.272			-6.423			-6.009		
<i>APOE</i>			164			88			88
ε2	-0.451	0.151 <sup>c,d</sup>		1.096	0.567 <sup>c,d</sup>		1.948	0.263 <sup>c,d</sup>	
ε4	0.515			-0.571			0.494		

GCR global clinical rating, SYP synaptophysin, MAP 2 microtubule-associated protein-2

<sup>a</sup> All loci except *MBL* and *APOE* were run under a dominant acting genetic model

<sup>b</sup> Log10 plasma HIV-1 RNA viral load and race/ethnicity included as covariates

<sup>c</sup> Omnibus *p* value

<sup>d</sup> ε2 by ε4 interaction not significant, so omitted from the model

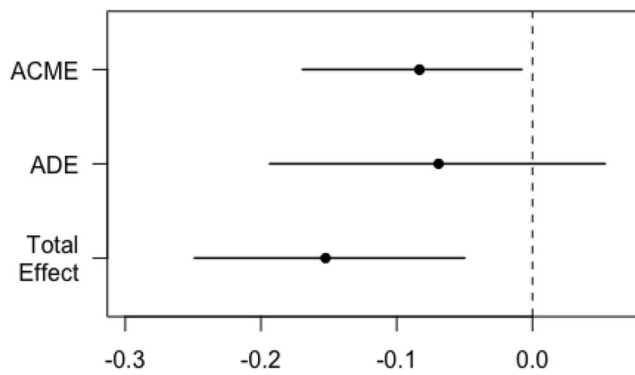
**Table 6** Effect sizes and *p* values of GCR, MAP 2, and SYP as outcomes

Outcome	Log10 plasma HIV-1 RNA viral load	<i>p</i> value	Race/ ethnicity <sup>a</sup>	<i>p</i> value	SYP	<i>p</i> value	MAP 2	<i>p</i> value
GCR	0.171	0.235	-0.862 -0.491 0.379	0.314 <sup>b</sup>	-0.068	0.280	-0.146	0.028
MAP 2	-0.056	0.812	-0.258 0.183 1.725	0.886 <sup>b</sup>	0.618	3.37 × 10 <sup>-11</sup>	-	-
SYP	-0.350	0.149	0.991 -0.362 -1.532	0.363 <sup>b</sup>	-	-	0.645	3.37 × 10 <sup>-11</sup>

GCR global clinical rating, MAP 2 microtubule-associated protein-2, SYP synaptophysin

<sup>a</sup> Categorized as African American, Hispanic, Asian/Native Alaskan with Caucasian as the reference group

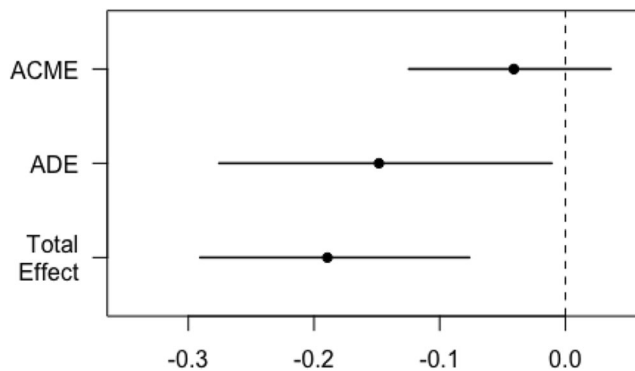
<sup>b</sup> Omnibus *p* value



**Fig. 1** Mediation analysis results for the model MAP 2 mediating the effect of SYP on GCR. The model includes log<sub>10</sub> plasma HIV-1 RNA viral load and race/ethnicity as covariates. Estimates for the average causal mediation effect (ACME), the average direct effect (ADE), and the total effect are denoted as closed circles, and the 95% confidence intervals (CI) are denoted as horizontal lines. Effects significant at a *p* value less than 0.05 do not cross the hatched vertical line at zero. The ACME *p* value = 0.032, the ADE *p* value = 0.282, and the total effect *p* value = 0.004. The proportion mediated = 0.547 (95% CI = 0.045, 1.832; *p* value = 0.036), supporting the hypothesis that MAP 2 mediates the effect of SYP on GCR, SYP → MAP 2 → GCR (MAP 2 mediating SYP effect on GCR), <sup>a</sup> Controlling for log<sub>10</sub> plasma HIV-1 RNA viral load and race/ethnicity

greatest predictors of neurocognitive impairment (Saloner et al., 2019; Sacktor et al., 2016; Lake et al., 2017; Wu et al., 2018; Vance et al., 2016). As such, the documented adverse effects of the recreational drugs included in our analyses, as well perhaps of depression, may have been overshadowed by factors that were not measured.

The second step involved determining which histopathological markers were associated with GCR. Only MAP 2 and



**Fig. 2** Mediation analysis results for the model SYP mediating the effect of MAP 2 on GCR. The model includes log<sub>10</sub> plasma HIV-1 RNA viral load and race/ethnicity as covariates. Estimates for the average causal mediation effect (ACME), the average direct effect (ADE), and the total effect are denoted as closed circles, and the 95% confidence intervals (CI) are denoted as horizontal lines. Effects significant at a *p* value less than 0.05 do not cross the hatched vertical line at zero. The ACME *p* value = 0.296, the ADE *p* value = 0.030, and the total effect *p* value = 0.002. The proportion mediated = 0.212 (95% CI = -0.196, 0.871; *p* value = 0.294), indicating a lack of support for the hypothesis that SYP mediates the effect of MAP 2 on GCR, MAP 2 → SYP → GCR (SYP mediating MAP 2 effect on GCR), <sup>a</sup> Controlling for log<sub>10</sub> plasma HIV-1 RNA viral load and race/ethnicity

SYP were found to be significant predictors of global cognitive functioning. This finding is consistent with our earlier study (Levine et al., 2015), as well as other studies, many of which included samples from the NNTC (Moore et al., 2006; Bryant et al., 2017; Masliah et al., 1992). There are several mechanisms through which HIV-1 may affect synaptodendritic functioning, including direct actions via envelope protein gp120 and transactivator of transcription (tat) protein, as reviewed in (Wenzel et al., 2019). Furthermore, our inclusion of potential confounding variables found to be significant in the initial step of our analyses revealed that plasma HIV-1 RNA viral load was significantly associated with SYP, but not with MAP 2.

In the third step, we tested for associations of each candidate genetic marker with GCR, SYP, and MAP 2, controlling for plasma HIV-1 RNA viral load and race/ethnicity. We expected to discover some associations, based on our previous finding (Levine et al., 2015). However, there were no significantly associated genetic markers with any of the outcomes after correcting for multiple comparisons using an FDR of 0.05. Therefore, despite the increase in sample size from the previous study, we found no statistically significant association of these outcomes with the genetic markers, suggesting that these genetic pathways are not major determinants of neurocognitive dysfunction or neurodegeneration among PLWH with advanced disease. Such findings further underscore the likely lack of consistently significant genetic influence on neurobehavioral outcomes in PLWH, as reviewed in (Levine et al., 2014a; Kallianpur & Levine, 2014).

The final step of our study was to apply mediation analysis to examine potential causal pathways relating SYP, MAP 2, and GCR. This step was taken to determine if the effect of MAP 2 on GCR was mediated through SYP, or if the effect of SYP on GCR was mediated through MAP 2. As the directed pathway of MAP 2 to SYP to GCR did not show any significant evidence and the directed pathway of SYP to MAP 2 to GCR was significant, the most plausible causal pathway is that MAP 2 mediates the effect of SYP on GCR. Coupled with the findings from earlier steps, an overall model emerges in which elevated plasma HIV-1 RNA viral load results in presynaptic degeneration (as indicated by SYP levels), which in turn leads to somatodendritic degeneration (as indicated by MAP 2 levels) and ultimately neurocognitive dysfunction. However, even if presynaptic degeneration is a downstream effect of more active systemic viral replication, it is almost certain that additional intermediary factors are involved, such as changes in neurogranin and calmodulin (Guha et al., 2018), cathepsin B and serum amyloid p component (Cantres-Rosario et al., 2019), E2F transcription factor-3 (Santerre et al., 2019), and *BCL11B*-encoded protein (Desplats et al., 2013), to name just a few. Importantly, this putative multicomponent sequence of events leading to neurocognitive dysfunction in PLWH is just one of a list of several other causes, a list

that includes medical comorbidities (Saloner et al., 2019; Yu et al., 2019), low-grade systemic chronic inflammation (Geffin & McCarthy, 2018), and antiretroviral medication toxicity (Tovar-y-Romo et al., 2012; Gannon et al., 2017).

The current study had several limitations. Perhaps most important was the absence of key variables in our analysis, including those mentioned in the immediately preceding paragraph (e.g., medical comorbidities), as well as brain HIV-1 viral load, CD163, and germane medical comorbidities. Additionally, the generalizability of the findings to the current cART era is limited, as the NNTC cases typically have more advanced illness and comorbidities than the vast majority of PLWH, at least within the USA (Morgello et al., 2001). This is best demonstrated by the relatively high rate of HIV-associated dementia (29%) and HIV encephalitis (9%) in our sample. Our use of plasma HIV-1 RNA viral load rather than the brain- or CSF-derived viral load also limits the interpretability, as these two measures are not strongly correlated (Gelman et al., 2013; Bavaro et al., 2019). Finally, while our study may be among the largest genetic-histopathological studies of HIV-related neurocognitive impairment, the statistical power was nevertheless limited by the sample size.

In conclusion, while our results affirmed the role of synaptodendritic degeneration in HIV-related neurocognitive impairment, we did not find definitive evidence of a host genetic influence on these histopathological markers among individuals with advanced HIV-1 disease. Our study adds to the existing literature of genetic association studies of HAND, which have focused on behavioral rather than histopathological phenotypes. Our findings do suggest that presynaptic degeneration precedes somatodendritic degeneration in the lead up to neurocognitive impairment.

**Author contributions** All authors contributed to the study conception, design, and/or execution. Andrew Levine and David Moore were involved in the initial study conception and design. Asha Kallianpur was involved in selecting genetic markers for the study. Data and specimen collection were performed or overseen by David Moore and Elyse Singer at their respective National NeuroAIDS Tissue Consortium sites. Tissue analysis was performed by Virawudh Soontornniyomkij and Eliezer Masliah. Data analysis was performed by Janet Sinsheimer, Sarah, Ji, Steve Horvath, and Andrew Levine. The first draft of the manuscript was written by Andrew Levine, David Moore, Virawudh Soontornniyomkij, Janet Sinsheimer, and Sarah Ji, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflicts of interest.

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