Atrophic brain signatures of mild forms of neurocognitive impairment in virally suppressed HIV infection

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Objective: There is a lack of evidence for the neurobiological underpinning of asymptomatic neurocognitive impairment (ANI) and mild neurocognitive disorders (MNDs) in virally suppressed HIV-positive persons. We hypothesized that such mild impairment would be associated with focal brain atrophy.

Design: A cross-sectional observational study.

Methods: Eighty-five virally suppressed HIV-positive and 44 geographically, demographically and lifestyle comparable HIV-negative men underwent anatomical MRI, neuropsychological evaluation and HIV laboratory tests. Volumes of interest (VOI) from magnetic resonance (MR) images were extracted using FreeSurfer to yield grey and white matter volumes in regions associated with HIV-related brain injury. HIV-associated neurocognitive disorder (HAND) [ANI = 38%, MND = 13%, HIV-associated dementia (HAD) = 3% vs. neuropsychologically-normal] was classified using Global Deficit Score (GDS $\geq$0.5) and functional decline. Effects of HIV status on VOI were assessed with multivariate analyses controlling for family-wise error. HAND categories and HIV biomarker effects on VOI were assessed with multiple regression.

Results: Relative to the HIV-negative group, the HIV-positive group demonstrated subcortical grey ($d = 0.50–0.60$) and white matter ($d = 0.43–0.69$) atrophy, with relative cortical sparing ($d = 0.23$). ANI showed reduced medial-orbitofrontal white matter compared with NP-normal cases ($P = 0.04$). MND showed enlarged lateral ventricles ($P = 0.02$) and reduced caudal-middle-frontal white matter ($P = 0.04$), caudal-anterior-cingulate white matter ($P = 0.006$) and inferior-parietal white matter ($P = 0.04$) compared with neuropsychologically normal. Across the HIV-positive group, lower $CD4^+$/CD8 ratio was the strongest predictor of atrophy in subcortical regions. Across HAND categories, HIV disease duration uniquely predicted greater medial-orbitofrontal white matter atrophy only in ANI ($P = 0.002$).

Conclusion: ANI shows specific frontal white matter atrophy to which HIV disease duration is a unique contributor. MND is characterized by more widespread subcortical atrophy.

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Keywords: brain atrophy, cognition, HIV, HIV-associated neurocognitive disorder, neuroimaging, viral suppression, volumetric MRI

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Introduction

The degree of brain atrophy present in HIV-infected (HIV-positive) cohorts has been investigated with MRI, but the results are inconsistent due to heterogeneity in participants' demographic and clinical characteristics, and in the MRI processing/analytical platforms used [1]. When focusing on studies conducted in the combined antiretroviral therapy (cART) era (Supplemental Digital Content 1 presents a list of 32 studies and review, http://links.lww.com/QAD/B380), we found three primary methodological issues affecting the reliability of the reported atrophic changes in treated HIV-infected adults:

(1) Thirty percent of studies did not include a HIV-negative control group and another 20% included controls that were significantly different in terms of age, education, race/ethnicity, geographical location or lifestyle.
(2) HIV-associated neurocognitive disorders (HANDs) prevalence and clinically relevant categories of asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND) and HIV-associated dementia (HAD) have not been systematically reported. Neuropsychological testing was sometimes not conducted at all (five out of 32 studies). This is a significant limitation because the severity of HAND is a major predictor of the degree of subcortical grey matter, white matter and cortical grey matter atrophy in the majority of studies examining this effect. Furthermore, no study has carefully evaluated the difference between ANI and MND. This is important because there is debate as to whether ANI has no neurobiological underpinning and solely represents a statistical neuropsychological entity.
(3) There was a large variation in MRI processing/analytical platforms due to the vast selection of choices that researchers have at their disposal and a lack of uniform standards, as well as the continuous development of new tools and updated versions of existing ones. This affects the magnitude of the differences seen between controls and clinical groups [1]. Although it may be less of an issue in dementia populations [2,3], it represents a major problem for detecting clinically relevant differences between controls and clinical groups at the early stage of disease, which is typically characterized by mild neurocognitive deficits such as mild HAND. More concerning is that these methodological differences have led to wide variations between measures of cognition and disease and their association with MRI-derived volumes [2,3]. In our review of the literature presented in Supplemental Digital Content 1, http://links.lww.com/QAD/B380, we found that the degree of cortical atrophy in HIV-positive cohorts compared with controls is larger when using voxel-based morphometry (VBM) than non-VBM methods. As such, MRI analysis working in the native space (e.g. FreeSurfer) or gold-standard manual tracing of regions of interest (ROI) appear more conservative than VBM. Our review also shows that grey matter volume differences by HIV status are more consistently observed than cortical differences, although there are some exceptions. Moreover, although subcortical and cortical GM specific anatomical regions have been commonly measured, white matter has been typically considered as a unified volume without factoring the striatofrontal vulnerability to HIV-related brain injury. In this regards, cART era MRI studies included in our review (Supplemental Digital Content 1, http://links.lww.com/QAD/B380) continue to show a predominance of subcortico-frontal injury with more or less involvement of parietal and temporal regions depending on the degree of past immune compromise, and degree of cognitive impairment, with sparing of the most posterior and occipital regions.

This study aimed to address these shortcomings and provide an estimate of brain atrophy in virally suppressed HIV-positive persons with no impairment on neuropsychologically testing vs. mild forms of neurocognitive deficit (ANI and MND):

(1) By focusing on a suppressed HIV-positive cohort who were clinically stable;
(2) By including demographically, geographically and lifestyle comparable HIV-negative controls;
(3) By concentrating on volumes of interest (VOI) in specific brain regions known to be affected by HIV including specific white matter regions; and
(4) Using automated segmentation in the native space for delineating individual anatomical ROI.

Finally, we examined the effects of a range of HIV disease biomarkers on the degree of atrophic changes in the entire HIV-positive sample and in the HAND clinical categories.

Informed by our review presented in Supplemental Digital Content 1, we hypothesized that there would be statistically significant overall subcortical and white matter, but not cortical atrophy in the HIV-positive group compared with HIV-negative controls. We hypothesized that grey matter and white matter atrophy could be observed in ANI, but would be more extensive in MND. We predicted that lower nadir $CD4^+$ cell count, longer HIV duration and lower $CD4^+/CD8^+$ ratio would be associated with a greater degree of subcortical and frontal white matter atrophy particularly in MND.

Materials and methods

Participants

Ninety-two HIV-positive and 45 HIV-negative individuals aged more than 45 years completed the MRI and neuropsychological examinations of the HIV and Brain Aging Research Program between 2009 and 2012. The sample inclusion/exclusion criteria have been described in detail previously [4,5]. In the case of this study, we also

excluded cases with a pacemaker, unsafe MRI implants and claustrophobia ($n = 5$), cases with movement artefacts not resolved by movement correction ($n = 2$) and extreme subcortical atrophy that the FreeSurfer 5.3 algorithm could not resolve ($n = 1$). The final samples included 85 HIV-positive and 44 HIV-negative individuals.

Study procedure and measures

MRI acquisition

MRI scans were acquired at St. Vincent's Hospital, Sydney Medical Imaging Department on a Phillips 3T Achieva TX scanner (Philips Medical Systems, Best, The Netherlands) using an eight-channel head coil. Two 3D T1-weighted anatomical images were obtained in the coronal plane (TFE: TR/TE: 6.39/2.9 ms, flip angle: 8°; FOV 256 mm; 190 slices, 1 mm isotropic). For one participant, only one T1 image was collected to minimize potential movement artefact from discomfort. A single T2-weighted image was also acquired in the axial plane (FLAIR: TR/TE: 11000/110 ms, TI: 2800 ms, flip angle: 90°; FOV 250 mm, 38 slices, 3.5 mm). FLAIR images were used to correct the segmentation of white matter hyperintensities, particularly on the posterior horn of the lateral ventricles.

MRI processing

FreeSurfer v5.3 software (https://surfer.nmr.mgh.harvard.edu/) [6] was used to process T1-weighted images by segmenting brain structures and regions into relevant neuroanatomical parts [6–8] (see Supplemental Digital Content 2, http://links.lww.com/QAD/B380 for more details and illustrations of brain segmentation). FreeSurfer was selected, as it is the only automated segmentation protocol that has been systematically assessed against gold-standard manual tracing in a large, multisite HIV-positive sample, which found it to be a robust method particularly for subcortical volumes [9]. FreeSurfer has been shown to overestimate the smaller subcortical structures in non-HIV samples [10,11], although this effect is most often seen in elderly participants (>75 years old) [12] who were not recruited for this study. Nevertheless, HIV-related brain injury may accelerate brain changes such that a HIV-positive 50 year-old may appear equivalent to a HIV-negative person aged 75 years. To minimize these potential issues [9], all FreeSurfer outputs were carefully reviewed blind to HIV status by MN following the FreeSurfer recommended corrections protocol (Supplemental Digital Content 2, http://links.lww.com/QAD/B380).

Volumes of interest

Global brain volumes and brain regions shown to be primarily affected by HIV in the cART era [13–15] were determined *a priori*, including total cortical volume, total white matter volume, frontal and parietal grey matter, all basal ganglia nuclei, lateral ventricles, fronto-striatal, fronto-parietal white matter and grey matter (Supplemental Digital Content 2, http://links.lww.com/QAD/B380). Each VOI was expressed as a percentage of estimated total intracranial volume (eTIV). We selected a ROI analysis to optimize the accuracy of brain atrophy estimates in our HIV-positive sample, as this procedure is more standardized than whole-brain methods such as VBM [2].

Neuropsychological assessment and determination of HIV-associated neurocognitive disorder clinical categories and laboratory visit

The standard neuropsychologically battery used in this cohort has been detailed previously [4,5]. It evaluated seven cognitive domains, functional independence in activities of daily living, mood status, and medication adherence. The HAND clinical categories of ANI, MND and HAD were classified using Global Deficit Score (GDS) and functional data as outlined in Cysique *et al.* [4]. Details of the laboratory visit were also reported in that article and included all biomarkers listed in Table 1.

Statistical analysis

To minimize the total number of statistical comparisons, we used the following strategy. First, between-group (HIV status) analyses of variance (ANOVAs) were conducted for the global volumes: cortex, total white matter and subcortical grey matter. We found that total white matter ($P < 0.004$) and subcortical GM ($P < 0.007$) were smaller in the HIV-positive group than the HIV-negative group, but not the cortex ($P = 0.22$). The subsequent analyses therefore focused on white matter and subcortical grey matter. Briefly, the HIV-positive group had smaller inferior-temporal cortex than the HIV-negative group ($P = 0.005$) and there were no other differences. We note that none of these cortical regions statistically differed between HIV-positive neuropsychologically normal, ANI and MND except for a trend for lower VOI in MND compared with ANI and

Table 1. Disease characteristics of HIV-positive group.

Biomarkers	Median	Range
Median HIV duration (years)	20	4.5–30.6
Median current cART duration (months)	26	6–156
Median nadir $CD4^+$ cell count (cells/μl)	185	0–350
Median current $CD4^+$ cell count (cells/μl)	528	77–1476
Asymptomatic neurocognitive impairment (ANI)	38%	–
Mild neurocognitive disorder (MND)	13%	–
HIV-associated dementia (HAD)	3%	–
Historical AIDS[a]	71%	–
Plasma HIV RNA undetectable (<50 copies/ml) (%)	98%	–
CSF HIV RNA undetectable CSF (<50 copies/ml) (%)[b]	97%	–

Continuous data presented as Mean (SD) and range unless otherwise specified. Percentage values provided for categorical data.1. Virtually, all participants were virally suppressed in plasma at baseline; the two detectable cases were 'viral blips' who were undetectable both prior to and poststudy.

[a]AIDS classification based on Centre for Disease Control and Prevention (CDC) 1993 HIV Disease Staging System.

[b]$n = 37$ participants consented to lumbar puncture for cerebrospinal fluid (CSF) analysis.

neuropsychologically normal in the inferior-parietal cortex ($P = 0.10$). Cortical parcellated volumes analyses are provided in Supplemental Digital Content 3, http://links.lww.com/QAD/B380.

Second, white matter and subcortical grey matter VOIs for the HIV-positive and HIV-negative groups were compared using multivariate analysis of variance (MANOVA) allowing corrections for family-wise error rate. MANOVA is a robust statistical technique when the magnitude of correlations between dependent variables (i.e. VOI) remains between ± 0.6 [16] as is the case with our dataset. Following this rationale, the nucleus accumbens was excluded after finding no between-group difference (i.e. $d = 0$). The MANOVA included 18 VOI in the final models: lateral ventricle, caudate, putamen, pallidum, thalamus, hippocampus, amygdala, caudal-anterior-cingulate white matter, caudal-middle-frontal white matter, cuneus, inferior-parietal white matter, inferior-temporal white matter, superior-frontal white matter, superior-parietal white matter, superior-temporal white matter, isthmus-cingulate white matter, medial-orbitofrontal white matter and insula volume. An age and HIV interaction effect was added to this model. Age was defined as age 60 vs. 60+ years.

Next, we assessed the effects of HAND clinical categories and HIV biomarkers on white matter and subcortical grey matter VOI. ANI ($n = 32$) and MND ($n = 10$) were compared with neuropsychologically normal cases ($n = 40$), and HIV duration, nadir $CD4^+$ cell count, current $CD4^+$ and $CD8^+$ effects on VOI were assessed with multiple regression.

Analyses were conducted in JMP v13.0 (SAS Inc., Cary, North Carolina, USA) with statistical significance set at P value less than 0.05; Cohen's d effect sizes were also computed.

Results

Participant demographic and clinical characteristics are presented in Table 2, while the HIV disease and laboratory data are presented in Table 1.

HIV serostatus differences in volumes of interest

The overall HIV status MANOVA model test was significant [Pillai's Trace, Wilks' lambda, Hotelling's Trace and Roy's Largest Root $F(19,109) = 2.108$; $P < 0.01$]. All volumes were smaller in the HIV-positive group than HIV-negative controls (Fig. 1). Medium to large effect sizes ($ds = 0.50–0.69$) were detected principally in the subcortical grey matter and white matter (Fig. 2). There was a small HIV * age interaction effect for the inferior-parietal WM ($P < 0.02$, $\eta^2 = 0.044$) indicating that those who were HIV-positive and aged 60+ had the smallest volumes.

Table 2. Demographic characteristics of the study samples.

	HIV-negative	HIV-positive	*P*
N	44	85	
Age (years)	53.9 (6.5)	54.9 (6.5)	0.42
Range	45–67	45–69	
Male (%)	100	100	–
Education (years)	15.1 (2.7)	14.0 (2.9)	0.05
Range	10–20	8–20	
Urban dwelling MSM (%)	84.1	89.3	.39

Continuous data presented as Mean (SD) and range unless otherwise specified. Percentage values provided for categorical data.

Asymptomatic neurocognitive impairment, mild neurocognitive disorders vs. neuropsychologically normal volumes of interest comparisons

ANI showed reduced medial-orbitofrontal white matter compared with NP-normal cases ($P = 0.04$, *Std* $\beta = -0.31$). MND showed enlarged lateral ventricles (*Std* $\beta = 0.34$, $P = 0.02$), reduced caudal-middle-frontal white matter (*Std* $\beta = -0.32$, $P = 0.04$), caudal-anterior-cingulate white matter (*Std* $\beta = -0.42$, $P = 0.006$), and inferior-parietal white matter (*Std* $\beta = -0.33$, $P = 0.04$) compared with neuropsychologically normal cases (Fig. 3).

HIV biomarkers

When considering the combined effect of HIV biomarkers across the HIV-positive sample, the overall model showed significant differences in the putamen ($\eta^2 = 0.139$, $P < 0.008$), amygdala ($\eta^2 = 0.098$, $P < 0.04$), lateral ventricles ($\eta^2 = 0.119$, $P < 0.02$) and superior-parietal white matter ($\eta^2 = 0.115$, $P < 0.02$). The effect size of the model's explanatory power was medium to large. There were no significant effects of nadir $CD4^+$ cell count on specific brain volumes. A medium effect size was found for longer HIV duration leading to smaller medial-orbitofrontal white matter ($\eta^2 = 0.071$, $P < 0.02$). Lower $CD4^+/CD8^+$ ratio was associated with medium-to-large reductions in putamen ($\eta^2 = 0.107$, $P < 0.004$) and superior-parietal white matter volumes ($\eta^2 = 0.084$, $P < 0.009$) as well as a small reduction in caudate volume ($\eta^2 = 0.054$, $P = 0.04$) and a small increase in lateral ventricle volume ($\eta^2 = 0.051$, $P < 0.04$) (Fig. 4).

When considering the effects of HIV biomarkers across the HAND clinical categories, we found that HIV disease duration uniquely predicted greater medial-orbitofrontal white matter atrophy only in ANI (*Std* $\beta = -0.51$, $P = 0.002$) (Figure S1 Supplemental Digital Content 4, http://links.lww.com/QAD/B380). Finally, no differences emerged between the ANI, MND and neuropsychologically normal cases on any biomarkers including a set of cardiovascular biomarkers collected as part of the study, aside from for a small-to-medium difference in $CD4^+/CD8^+$ ratio between MND and neuropsychologically normal ($P = 0.04$) (Table S1 Supplemental Digital Content 4, http://links.lww.com/QAD/B380).

In summary, we found:

(1) Relative to the HIV-negative group, the HIV-positive group showed moderate atrophy of subcortical white

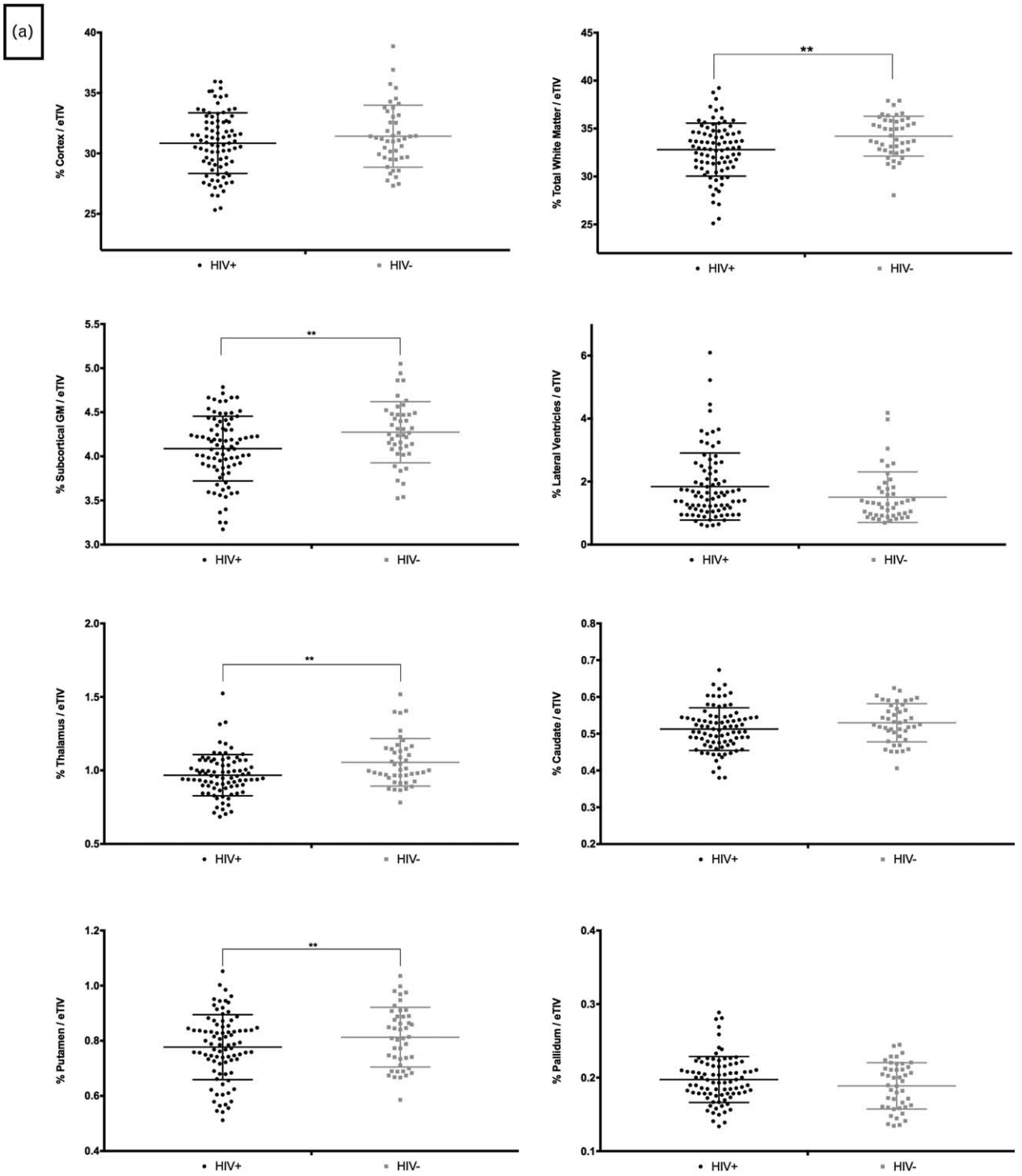

Fig. 1. (a–c) Brain volume comparisons between the HIV-negative and HIV-positive samples. *VOI (% eTIV)* mean and standard deviations are presented. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ controlled for multiple comparisons with MANOVA. The relative contributions of each hemisphere to VOIs differences were checked using *t*-tests, and no lateralization effects found. VOIs distributions were inspected visually and quantitatively via histograms, skewness and kurtosis, and Shapiro–Wilks tests ($P > 0.05$). The following VOIs were transformed to better approximate the normal distribution: lateral ventricle (Log_{10}); thalamus, insula, isthmus-cingulate WM and medial-orbitofrontal WM (inverse transformation), as well as caudal-middle-frontal, isthmus-cingulate and medial-orbitofrontal GM (Log_{10}).

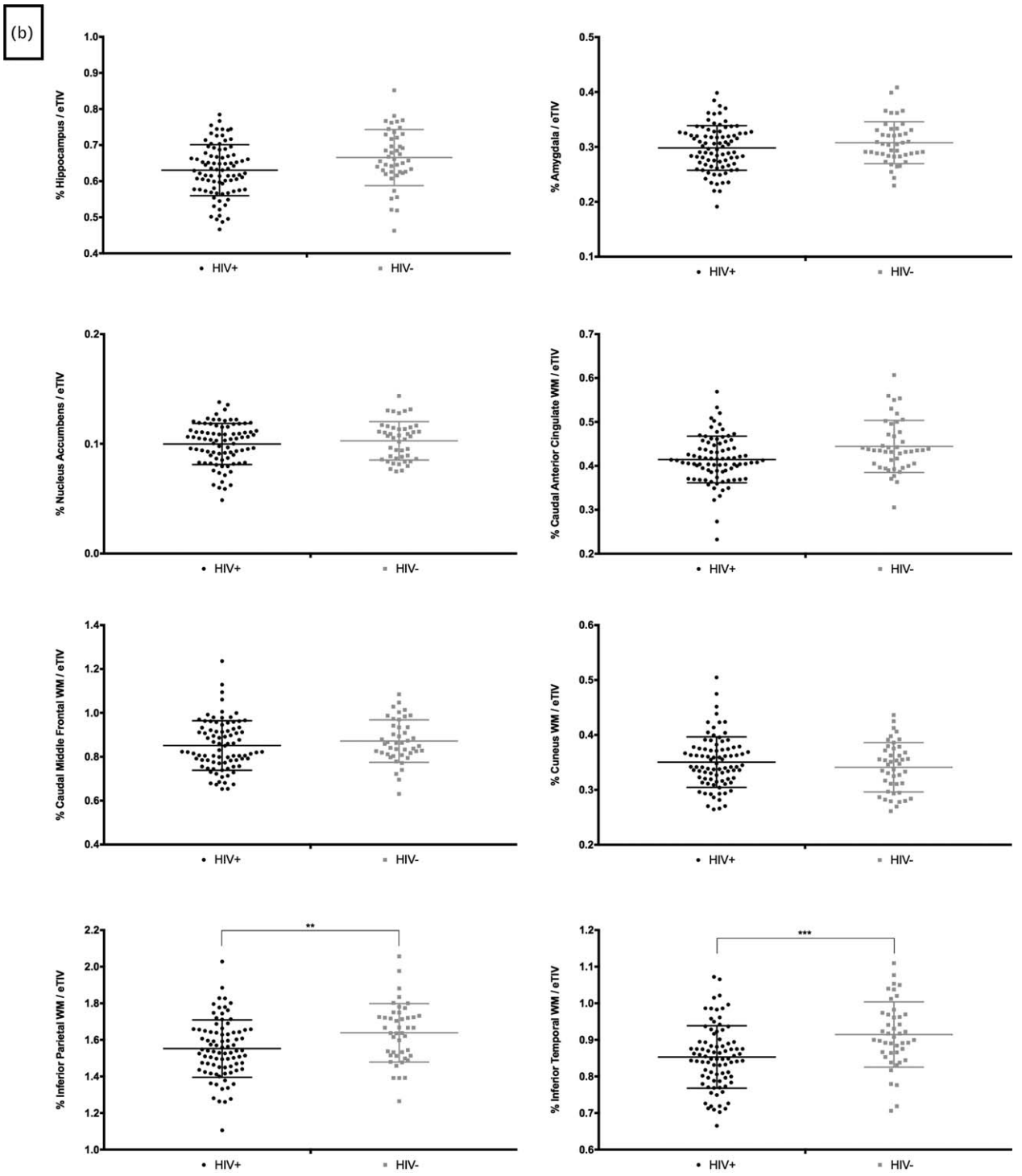

Fig. 1. (***Continued***).

matter tracts (inferior-temporal, inferior-parietal and caudal-anterior cingulate white matter) as well as subcortical grey matter atrophy (particularly for the thalamus and hippocampus). Furthermore, the HIV-positive group showed moderate cortical atrophy that was restricted to the inferior-temporal cortex.

(2) Compared with HIV-positive neuropsychologically normal cases, ANI cases showed moderate atrophy that was restricted to the medial-orbitofrontal white matter. By comparison, moderate white matter atrophy was more widespread in MND cases, including the caudal-middle-frontal white matter, caudal-anterior-cingulate

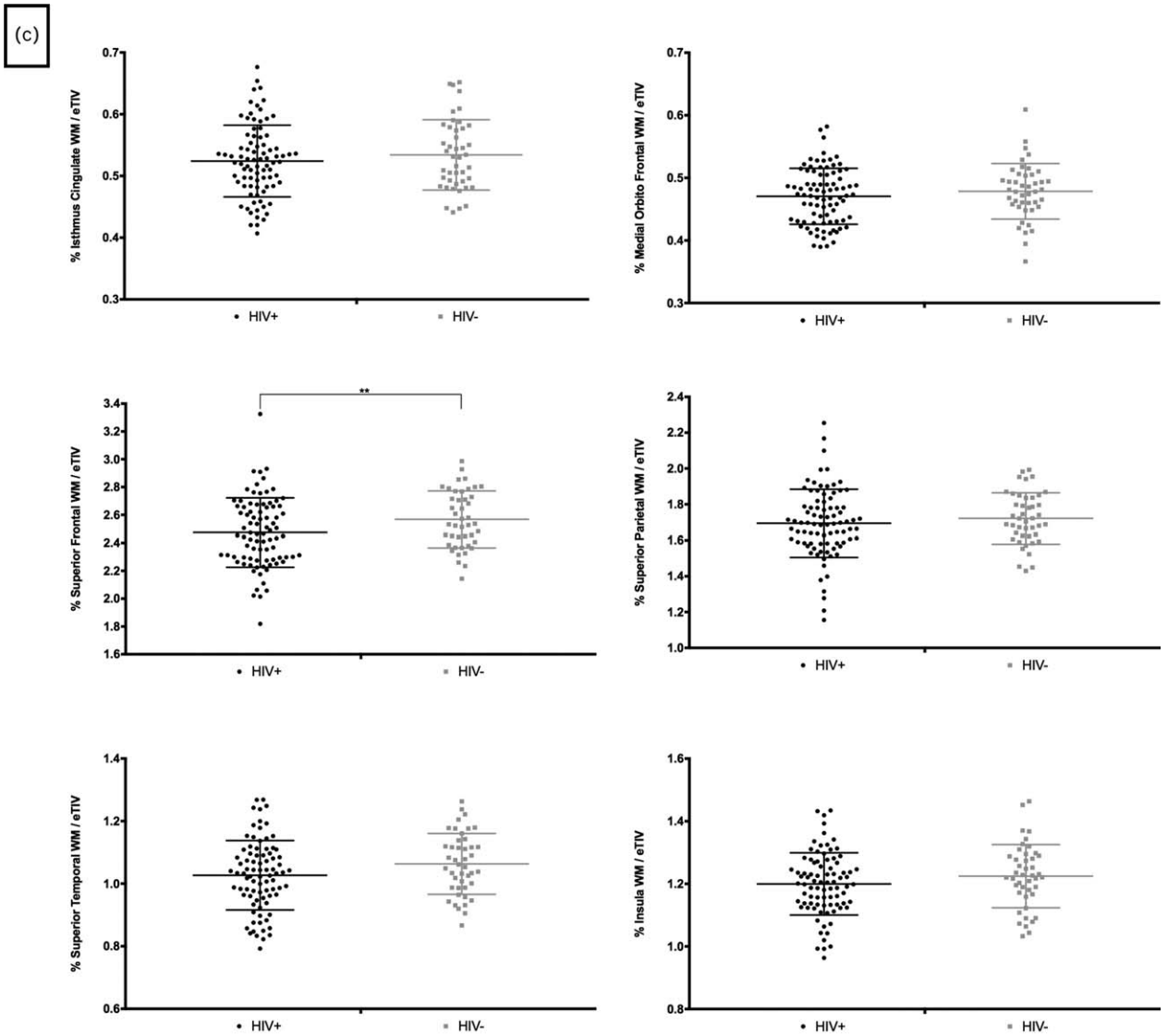

Fig. 1. (***Continued***).

white matter and inferior-parietal white matter. They also showed moderate lateral ventricular enlargement compared with the neuropsychologically normal cases.

(3) Longer HIV duration and HIV immune activation (defined as lower $CD4^+/CD8^+$ ratio) were associated with greater subcortical grey matter and white matter atrophy. Moreover, longer HIV duration independently predicted medial-orbitofrontal white matter only in the ANI cases.

(4) Age and HIV status interacted to produce greater inferior-parietal white matter atrophy in older HIV-positive persons.

Discussion

Our first finding of greater atrophy in subcortical brain regions than in cortical regions is in accordance with our hypothesis and confirms that structural abnormalities in subcortical grey matter and striato-frontal white matter regions persist despite viral suppression and stable cART in a cohort of chronically HIV-positive men [15,17,18]. Of particular interest is that the inferior-temporal white matter, hippocampus and inferior-temporal cortex are the most abnormally reduced. The involvement of these regions, known to be associated with Alzheimer's disease, is concerning [19]. It should be noted that no HIV disease biomarkers or demographic effects predicted atrophy in these regions. Furthermore, the HIV status × age interaction effect was restricted to one white matter region, possibly because our sample is still relatively young (only 30% over 60 years old).

Our study does not support previous findings of widespread reductions in cortical volumes in HIV-positive persons compared with controls [20–26]. One

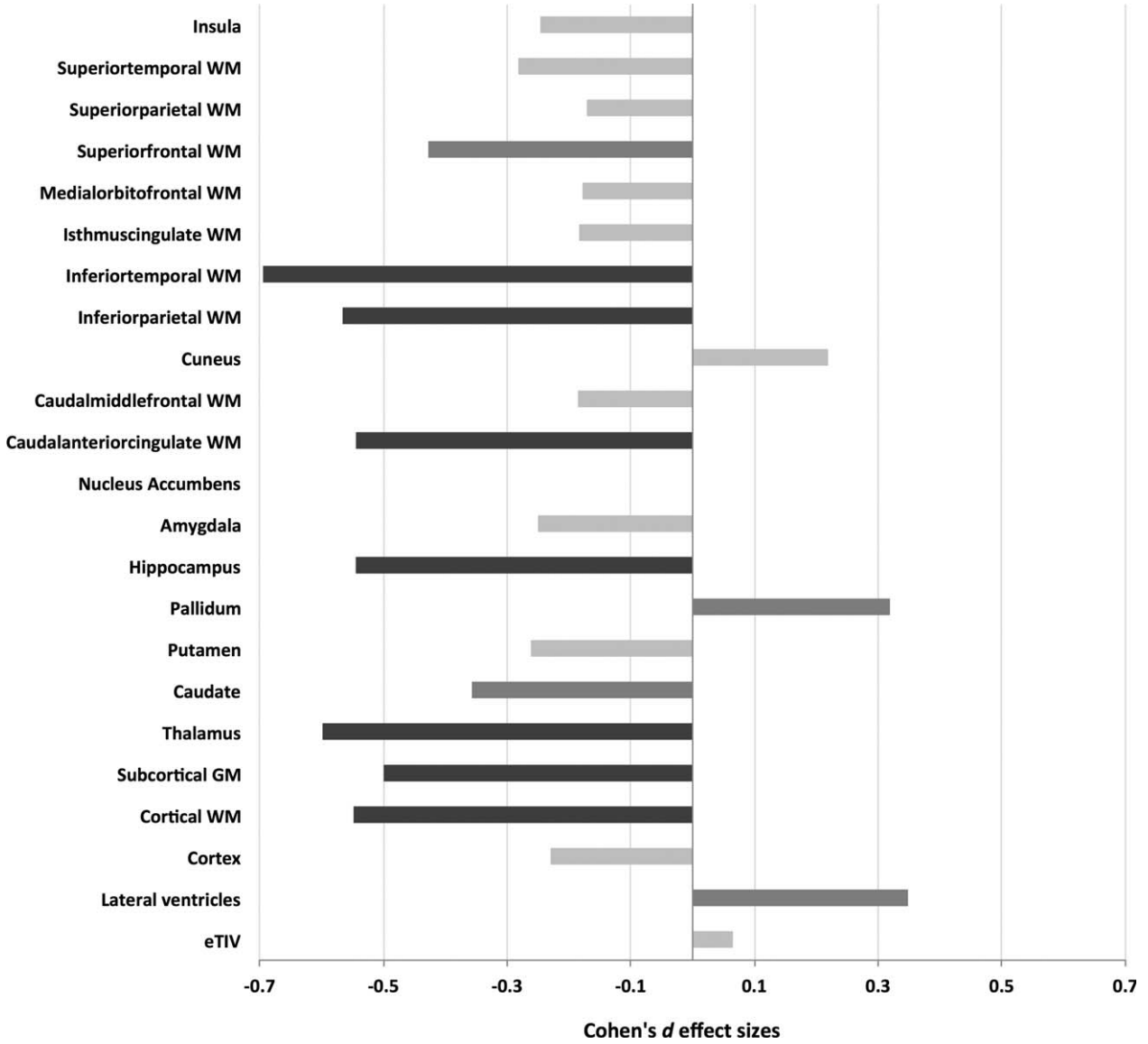

Fig. 2. Cohen's *d* effect sizes of volume differences between the HIV-negative and HIV-positive samples. Nucleus accumbens was not included due to effect size $d = 0$.

factor contributing to this disparity is the difference in MRI processing/analytical platform, wherein VBM-based analyses are more likely to find between-group cortical volume differences, although the magnitude of these effects is not always clear, as only significant results are typically reported. VBM overestimates grey matter differences compared with manual tracing [27] by potentially lumping together adjacent gyri around the corresponding sulci, causing falsely high cortical grey matter values [28]. Conversely, FreeSurfer is surface-based and segments entire subcortical regions based on a predefined atlas, independent of contrasts between tissue types [6].

ROI analyses have been criticised for not considering the entire brain. However, this is less of an issue when the disease in question has an established disease distribution, although its exact nature remains to be elucidated [29]. Further studies are needed comparing various platforms in NeuroHIV as has been done for other neurological disorders. In particular, more work is needed to determine the clinical relevance of different methods by assessing their association with HAND clinical categories.

The other explanation for discrepancies in the magnitude of overall cortical grey matter volume loss in our study may be that previous studies included small and/or, nondemographically comparable control samples. Doing so yields unreliable demographic effects that render any statistical adjustments problematic [16] and thereby affect the validity of VOI differences. In contrast, our HIV-negative controls were carefully selected to be geographically, lifestyle (i.e. urban MSM) and demographically

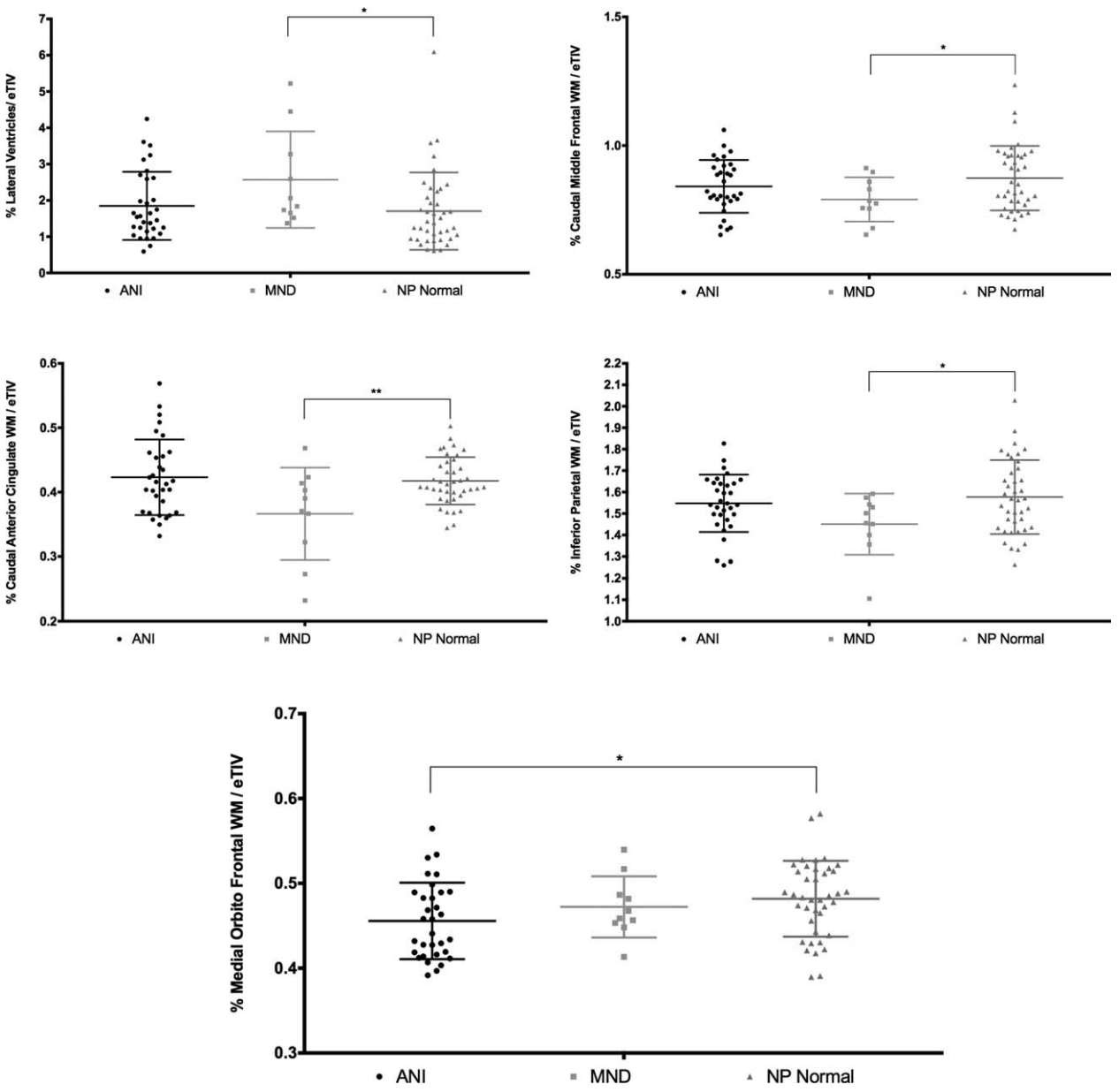

Fig. 3. Brain volume comparisons between ANI, MND and NP-Normal cases. *VOI (% eTIV)* mean and standard deviations are presented. NP-Normal cases were set a reference in Dunnett's control comparisons. $^{*}P < 0.05$; $^{**}P < 0.01$. ANI, asymptomatic neurocognitive impairment; MND, mild neurocognitive disorder.

(mostly well educated and high functioning) comparable to our HIV-positive sample. Our results empirically demonstrate the importance of including controls that closely resemble the specific demographics of local HIV-positive populations. The study [30] that used FreeSurfer and well matched HIV status groups with high levels of viral suppression also observed a more obvious subcortical-to-cortical atrophy gradient, congruent with our findings.

HIV-related brain injury targets the white matter [13]. However, past research lacks regional specificity with analyses often reporting reduced total brain white matter in HIV-positive participants without separately examining anatomically relevant structures and weakening any association with mild neurocognitive dysfunction. To the best of our knowledge, only one other study investigated HIV-related white matter atrophy in a similarly regionally specific manner [31], and found reductions in four corpus callosum regions in HIV-positive persons compared with controls. Moreover, greater cognitive impairment was associated with smaller isthmus and genu.

Our study reports regional volume differences as a function of HAND clinical categories. The finding that

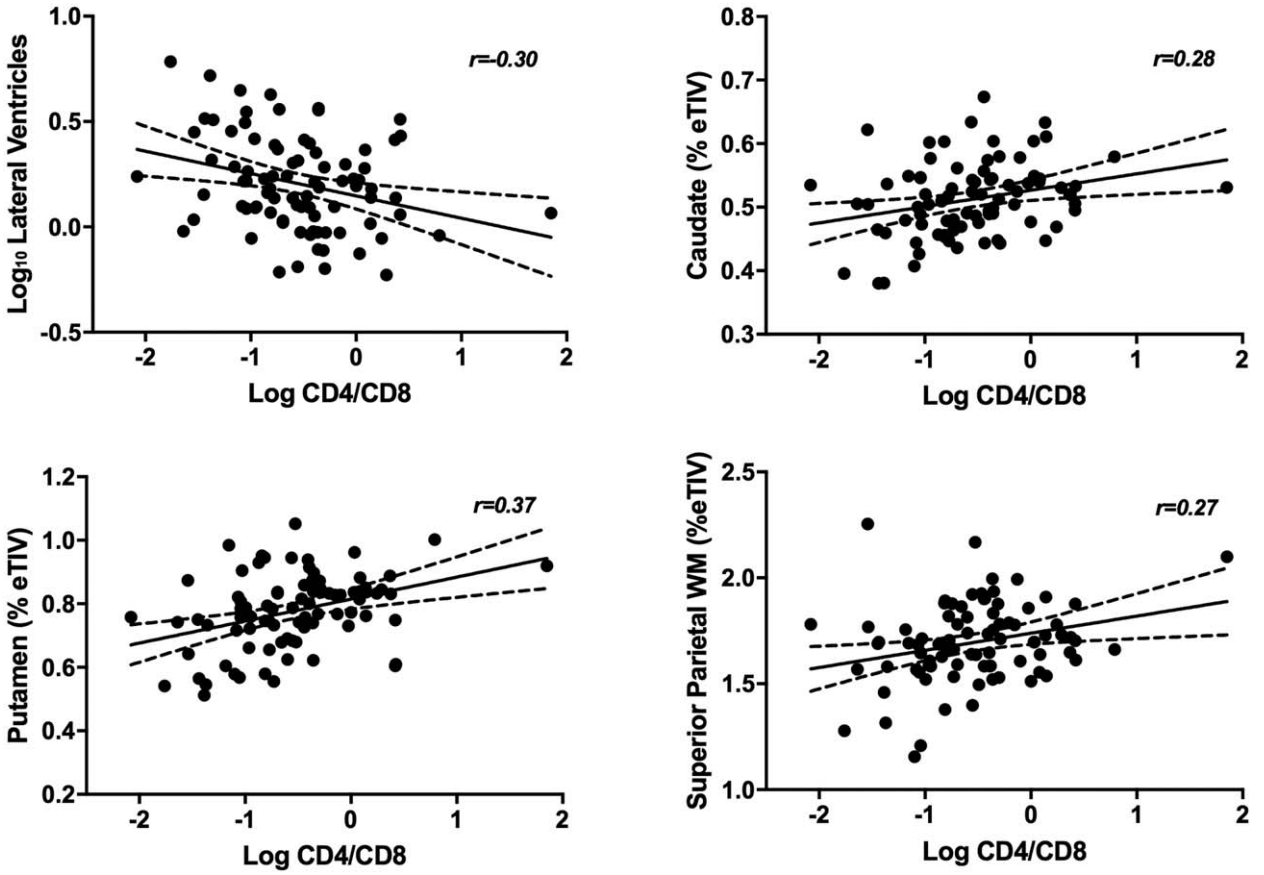

Fig. 4. A lower $CD4^+/CD8^+$ ratio is associated with volume loss across several subcortical grey and WM regions in the HIV-positive group. The Log $CD4^+/CD8^+$ ratio represents an independent effect on VOI in a model that also included the nadir $CD4^+$ cell count and HIV duration. For clarity of the illustration, the univariate relationships are represented.

ANI is associated with unique specific frontal white matter atrophy in the context of more widespread atrophy in MND suggests a potential neurobiological underpinning for ANI. Damage to subcortical white matter regions may account for the persistence of HAND in well controlled HIV infection and reflect at a neuroanatomical level a continuum of clinical disease progression in the brain, given that ANI are at a higher risk of progressing to symptomatic HAND [32]. In addition, the finding that HIV disease duration uniquely predicted ANI-related brain injury suggests that it may serve as a unique biomarker for ANI. Importantly, ANI, MND and neuropsychologically normal cases did not differ on any HIV disease or cardiovascular biomarkers (see Supplemental Digital Content 4), except for lower $CD4^+/CD8^+$ ratio in MND.

HIV clinical factors including nadir $CD4^+$ cell count and duration of infection have been inconsistently linked to specific volume reductions in the cART era. Lower nadir $CD4^+$ cell count has been associated with reduced posterior mid-body of the corpus callosum [31], total white matter [33,34] and subcortical grey matter volumes as well as increased cerebrospinal fluid volume [34], while we and others found no effect [18,35]. Range and variance in these biomarkers tend to affect the strength of such associations. Our study did not demonstrate a nadir effect, possibly because our range was restricted between 0 and 350 cp/ml by design, as this effect is fairly robust in studies containing a broader range of values.

Some studies have observed an association between longer HIV infection and smaller total grey matter [36], prefrontal grey matter [37] and basal ganglia [18], while the present study and others detected no effect at the HIV group level [31,33–35]. However, we uniquely detected this effect in ANI cases, further showing the importance of using standard diagnostic nomenclature in research.

$CD4^+/CD8+$ ratio was the strongest predictor of volume loss, with lower ratio associated with small and medium-large reductions in caudate, putamen, inferior-parietal white matter and superior-parietal white matter volumes as well as lateral ventricular enlargement. As the $CD4^+/CD8^+$ ratio is an indicator of HIV-related immune activation, this finding suggests that ongoing effects of

HIV disease despite treatment and viral suppression may be producing atrophic changes. Indeed, even with normalization of $CD4^+$ levels with cART, $CD4^+$/$CD8^+$ ratios remain low or inverted in many virally suppressed HIV-positive individuals [38]. The underlying mechanism by which peripheral HIV immune activation affects brain atrophy is not fully understood. However, pro-inflammatory cytokines, released by macrophages in response to persistent immune activation by HIV infection, are likely to be playing a role [39].

The present study has several limitations. Firstly, it should be recognized that participants were exclusively men with the age range restricted to more than 45 years. Larger studies are needed to extend the generalizability of our results to women and other international HIV-positive cohorts. Moreover, the cross-sectional nature of our design precluded the potential for monitoring neuroanatomical changes with progression of HIV-infection. In this regard, it is possible that the ANI difference to neuropsychologically normal that was not detected in the MND group is a trajectory-dependent effect linked to HIV duration. Longitudinal follow-up is needed to explore this effect further. Finally, in order to better elucidate HIV neuropathogenesis, more measures of immune activation should be considered, particularly central nervous system (CNS)-based biomarkers.

This study has shown evidence of atrophic brain changes of medium-large magnitude in subcortical and associated white matter regions as well as mild cortical atrophy, despite successful HIV treatment and viral suppression. Atrophy of the medial-orbitofrontal white matter was specific to ANI, suggesting a neurobiological basis to the disorder and confirming the differential validity of MND. It will be imperative to explore the evolution of these atrophic changes over time in relation to clinical progression and other HIV and CNS biomarkers in virally suppressed and well treated HIV-positive cohorts.

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L.A.C., C.D.R. and B.J.B. conceptualized the study. L.A.C., C.D.R., B.J.B. and K.J.M. contributed to study design. L.A.C. and B.J.B. oversaw participant recruitment. K.J.M. oversaw MRI data acquisition. L.A.C. conducted the majority of neuropsychological testing. T.M.G. conducted some neuropsychological testing under supervision of L.A.C. B.J.B. oversaw collection of laboratory data. J.R.S. inspected all MRI images for movement and artefact as part of his Honour's dissertation. M.J.N. completed MRI automated brain segmentation using FreeSurfer under supervision of L.A.C. and C.D.R. and conducted statistical analyses under supervision of L.A.C. as part of her Honour's dissertation. T.M.G. wrote the first draft of manuscript. All authors provided critical revisions to subsequent drafts.

Conflicts of interest

M.J.N., J.R.S. and K.J.M. report no disclosures.

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References

1. O'Connor EE, Zeffiro TA, Zeffiro TA. **Brain structural changes following HIV infection: meta-analysis.** *AJNR Am J Neuroradiol* 2018; **39**:54–62.
2. Callaert DV, Ribbens A, Maes F, Swinnen SP, Wenderoth N. **Assessing age-related gray matter decline with voxel-based morphometry depends significantly on segmentation and normalization procedures.** *Front Aging Neurosci* 2014; **6**:124.
3. Popescu V, Schoonheim MM, Versteeg A, Chaturvedi N, Jonker M, Xavier de Menezes R, *et al*. **Grey matter atrophy in multiple sclerosis: clinical interpretation depends on choice of analysis method.** *PLoS One* 2016; **11**:e0143942.
4. Cysique LA, Heaton RK, Kamminga J, Lane T, Gates TM, Moore DM, *et al*. **HIV-associated neurocognitive disorder in Australia: a case of a high-functioning and optimally treated cohort and implications for international neuroHIV research.** *J Neurovirol* 2014; **20**:258–268.
5. Lane TA, Moore DM, Batchelor J, Brew BJ, Cysique LA. **Facial emotional processing in HIV infection: relation to neurocognitive and neuropsychiatric status.** *Neuropsychology* 2012; **26**:713–722.
6. Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, *et al*. **Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain.** *Neuron* 2002; **33**:341–355.

7. Desikan RS, Segonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, *et al*. **An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest.** *Neuroimage* 2006; **31**:968–980.
8. Fischl B, Dale AM. **Measuring the thickness of the human cerebral cortex from magnetic resonance images.** *Proc Natl Acad Sci U S A* 2000; **97**:11050–11055.
9. Dewey J, Hana G, Russell T, Price J, McCaffrey D, Harezlak J, *et al*. **Reliability and validity of MRI-based automated volumetry software relative to auto-assisted manual measurement of subcortical structures in HIV-infected patients from a multisite study.** *Neuroimage* 2010; **51**:1334–1344.
10. Morey RA, Petty CM, Xu Y, Hayes JP, Wagner HR 2nd, Lewis DV, *et al*. **A comparison of automated segmentation and manual tracing for quantifying hippocampal and amygdala volumes.** *Neuroimage* 2009; **45**:855–866.
11. Perlaki G, Horvath R, Nagy SA, Bogner P, Doczi T, Janszky J, *et al*. **Comparison of accuracy between FSL's FIRST and Freesurfer for caudate nucleus and putamen segmentation.** *Sci Rep* 2017; **7**:2418.
12. Wenger E, Martensson J, Noack H, Bodammer NC, Kuhn S, Schaefer S, *et al*. **Comparing manual and automatic segmentation of hippocampal volumes: reliability and validity issues in younger and older brains.** *Hum Brain Mapp* 2014; **35**:4236–4248.
13. Ellis R, Langford D, Masliah E. **HIV and antiretroviral therapy in the brain: neuronal injury and repair.** *Nat Rev Neurosci* 2007; **8**:33–44.
14. O'Donnell LA, Agrawal A, Jordan-Sciutto KL, Dichter MA, Lynch DR, Kolson DL. **Human immunodeficiency virus (HIV)-induced neurotoxicity: roles for the NMDA receptor subtypes.** *J Neurosci* 2006; **26**:981–990.
15. Kuhn T, Schonfeld D, Sayegh P, Arentoft A, Jones JD, Hinkin CH, *et al*. **The effects of HIV and aging on subcortical shape alterations: a 3D morphometric study.** *Hum Brain Mapp* 2017; **38**:1025–1037.
16. Tabachnick BG, Fidell LS. Using multivariate statistics. 5th ed. New York: Allyn and Bacon; 2007.
17. Ances BM, Ortega M, Vaida F, Heaps J, Paul R. **Independent effects of HIV, aging, and HAART on brain volumetric measures.** *J Acquir Immune Defic Syndr* 2012; **59**:469–477.
18. Becker JT, Sanders J, Madsen SK, Ragin A, Kingsley L, Maruca V, *et al*. **Subcortical brain atrophy persists even in HAART-regulated HIV disease.** *Brain Imaging Behav* 2011; **5**:77–85.
19. Milanini B, Valcour V. **Differentiating HIV-associated neurocognitive disorders from Alzheimer's disease: an emerging issue in geriatric NeuroHIV.** *Curr HIV/AIDS Rep* 2017; **14**:123–132.
20. Becker JT, Maruca V, Kingsley LA, Sanders JM, Alger JR, Barker PB, *et al*. **Factors affecting brain structure in men with HIV disease in the post-HAART era.** *Neuroradiology* 2012; **54**:113–121.
21. Guha A, Brier MR, Ortega M, Westerhaus E, Nelson B, Ances BM. **Topographies of cortical and subcortical volume loss in HIV and aging in the cART era.** *J Acquir Immune Defic Syndr* 2016; **73**:374–383.
22. Sanford R, Fernandez Cruz AL, Scott SC, Mayo NE, Fellows LK, Ances BM, *et al*. **Regionally specific brain volumetric and cortical thickness changes in HIV-infected patients in the HAART era.** *J Acquir Immune Defic Syndr* 2017; **74**:563–570.
23. Underwood J, Cole JH, Leech R, Sharp DJ, Winston A. **Multivariate pattern analysis of volumetric neuroimaging data and its relationship with cognitive function in treated HIV-disease.** *J Acquir Immune Defic Syndr* 2018; **78**:429–436.
24. Sanford R, Fellows LK, Ances BM, Collins DL. **Association of brain structure changes and cognitive function with combination antiretroviral therapy in HIV-positive individuals.** *JAMA Neurol* 2018; **75**:72–79.
25. van Zoest RA, Underwood J, De Francesco D, Sabin CA, Cole JH, Wit FW, *et al*. **Structural brain abnormalities in successfully treated HIV infection: associations with disease and cerebrospinal fluid biomarkers.** *J Infect Dis* 2017; **217**:69–81.
26. Underwood J, Cole JH, Caan M, De Francesco D, Leech R, van Zoest RA, *et al*. **Gray and white matter abnormalities in treated human immunodeficiency virus disease and their relationship to cognitive function.** *Clin Infect Dis* 2017; **65**:422–432.
27. Kennedy KM, Erickson KI, Rodrigue KM, Voss MW, Colcombe SJ, Kramer AF, *et al*. **Age-related differences in regional brain volumes: a comparison of optimized voxel-based morphometry to manual volumetry.** *Neurobiol Aging* 2009; **30**:1657–1676.
28. Rajagopalan V, Pioro EP. **Disparate voxel based morphometry (VBM) results between SPM and FSL softwares in ALS patients with frontotemporal dementia: which VBM results to consider?** *BMC Neurol* 2015; **15**:015–0274.
29. Gelman BB. **Neuropathology of HAND with suppressive antiretroviral therapy: encephalitis and neurodegeneration reconsidered.** *Curr HIV/AIDS Rep* 2015; **12**:272–279.
30. Kallianpur KJ, Shikuma C, Kirk GR, Shiramizu B, Valcour V, Chow D, *et al*. **Peripheral blood HIV DNA is associated with atrophy of cerebellar and subcortical gray matter.** *Neurology* 2013; **80**:1792–1799.
31. Tate DF, Sampat M, Harezlak J, Fiecas M, Hogan J, Dewey J, *et al*. **Regional areas and widths of the midsagittal corpus callosum among HIV-infected patients on stable antiretroviral therapies.** *J Neurovirol* 2011; **17**:368–379.
32. Grant I, Franklin DR Jr, Deutsch R, Woods SP, Vaida F, Ellis RJ, *et al*. **Asymptomatic HIV-associated neurocognitive impairment increases risk for symptomatic decline.** *Neurology* 2014; **82**:2055–2062.
33. Hua X, Boyle CP, Harezlak J, Tate DF, Yiannoutsos CT, Cohen R, *et al*. **Disrupted cerebral metabolite levels and lower nadir CD4 + counts are linked to brain volume deficits in 210 HIV-infected patients on stable treatment.** *Neuroimage Clin* 2013; **3**:132–142.
34. Jernigan TL, Archibald SL, Fennema-Notestine C, Taylor MJ, Theilmann RJ, Julaton MD, *et al*. **Clinical factors related to brain structure in HIV: the CHARTER study.** *J Neurovirol* 2011; **17**:248–257.
35. Clark US, Walker KA, Cohen RA, Devlin KN, Folkers AM, Pina MJ, *et al*. **Facial emotion recognition impairments are associated with brain volume abnormalities in individuals with HIV.** *Neuropsychologia* 2015; **70**:263–271.
36. Cohen RA, Harezlak J, Gongvatana A, Buchthal S, Schifitto G, Clark U, *et al*. **Cerebral metabolite abnormalities in human immunodeficiency virus are associated with cortical and subcortical volumes.** *J Neurovirol* 2010; **16**:435–444.
37. Kuper M, Rabe K, Esser S, Gizewski ER, Husstedt IW, Maschke M, *et al*. **Structural gray and white matter changes in patients with HIV.** *J Neurol* 2011; **258**:1066–1075.
38. Serrano-Villar S, Gutierrez C, Vallejo A, Hernandez-Novoa B, Diaz L, Abad Fernandez M, *et al*. **The CD4/CD8 ratio in HIV-infected subjects is independently associated with T-cell activation despite long-term viral suppression.** *J Infect* 2013; **66**:57–66.
39. Vassallo M, Durant J, Lebrun-Frenay C, Fabre R, Ticchioni M, Andersen S, *et al*. **Virologically suppressed patients with asymptomatic and symptomatic HIV-associated neurocognitive disorders do not display the same pattern of immune activation.** *HIV Med* 2015; **16**:431–440.