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Accelerated epigenetic aging in adolescents living with HIV is associated with altered development of brain structures

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Abstract

We recently demonstrated that adolescents perinatally infected with HIV-1 (PHIV+) have accelerated aging as measured by a highly accurate epigenetic biomarker of aging known as

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Author contributions

AJL, JH, DJS, and SH conceived of the study. AJL and JH are the PI's of the R21 which primarily funded this study. HZ is PI of the CTAAC study, from which most of the data were derived. SJH and SE carried out the statistical analysis. JF extracted the brain imaging data. JH wrote the first draft of the article. The remaining authors conceived of and aided with the CTAAC study, including collection of the DNA samples and phenotypic data. All authors helped in the interpretation of the findings and the write up of the article.

Conflicts of interest

All authors: No reported conflicts.

the epigenetic clock. However whether epigenetic age acceleration in PHIV+ impacts brain development at the macro and microstructural levels of brain anatomy has not been studied. We report on a cross sectional study of PHIV+ enrolled in the Cape Town Adolescent Antiretroviral Cohort (CTAAC). The Illumina Infinium Methylation EPIC array was used to generate DNA methylation data from the blood samples of 180 PHIV+ aged 9 to 12 years. The epigenetic clock software and method was used to estimate two measures, epigenetic age acceleration (AgeAccelerationResidual) and extrinsic epigenetic age acceleration (EEAA). Each participant underwent T1 structural magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI). In order to investigate the associations of chronological age, sex, epigenetic age acceleration and treatment variables (CNS penetration-effectiveness score (CPE)) of antiretroviral regimen on brain structure in PHIV+, we developed stepwise multiple regression models in R (version 3.4.3, 2017) including grey and white matter volumes, cortical thickness, cortical surface area, and DTI measures of white matter microstructural integrity. The mean DNAm age (16.01 years) of the participants was higher than their mean chronological age (10.77 years). Epigenetic age acceleration contributed more to regional alterations of brain volumes, cortical thickness, cortical surface areas and neuronal microstructure than chronological age, in a range of regions. CPE positively contributed to volume of the brain stem. Understanding the drivers of epigenetic age acceleration could lead to valuable insights into structural brain alterations, and the persistence of neurocognitive disorders in seen in PHIV+

Keywords

HIV; DNA methylation; perinatal HIV; epigenetic clock; MRI; DTI; brain imaging

Introduction

Epigenetic mechanisms such as DNA methylation (DNAm) act as an interface between the environment and the genome, and could therefore play a role in the development of brain structures in adolescents living with HIV(PHIV+)(Gapp et al. 2014). An age adjusted measure of DNAm age, known as epigenetic age acceleration, takes on positive or negative values in individuals who age faster or slower than expected based on chronological age(Horvath 2013). Chronological age has been shown to have a profound effect on DNA methylation levels(Horvath et al. 2012). As a result, several highly accurate epigenetic biomarkers of chronological age have been proposed(Horvath et al. 2013). These biomarkers use weighted averages of methylation levels at specific CpG sites to produce estimates of age (in units of years), referred to as "DNA methylation age" (DNAm age) or "epigenetic age". Further, the utility of the epigenetic clock method using various tissues and organs has been demonstrated in applications surrounding frailty(Breitling et al. 2016) and HIV infection(Horvath et al. 2015).

We recently demonstrated that PHIV+ is associated with epigenetic age acceleration in young adolescents and with poorer cognitive functioning in these adolescents(Horvath et al. 2018). CD4+ T cell counts were negatively correlated with epigenetic age acceleration in PHIV+ adolescents(Horvath et al. 2018). In addition, our analysis of antiretroviral therapy (ART) regimen revealed that adolescents who were on second or third line ART were more

likely to have accelerated aging(Horvath et al. 2018), suggesting even greater rapidity of aging among those failed first line treatment. Earlier work conducted by our team using diffusion tensor imaging (DTI), found that HIV infected adolescents displayed decreased fractional anisotropy (FA) and increased mean diffusivity (MD) compared to well matched HIV uninfected peers, indicating altered neuronal microstructure(Hoare et al. 2015). In addition we have demonstrated that PHIV+ have regional macrostructural decreases in cerebral gray matter volumes, cortical surface area and decreased gyrification compared to uninfected peers(Hoare et al. 2018). A range of factors, including ART treatment factors and accelerated aging as measured via DNAm, could be associated with damaged macro and microstructure in PHIV+. CNS penetration-effectiveness score (CPE) of ART represents an estimation of the penetration of ART into the CNS and has been associated with lower viral loads(Letendre et al. 2008; Letendre et al. 2010). CPE could contribute to the variance of CNS macro and microstructure in PHIV+.

In the current study, we aimed to apply a pan tissue epigenetic clock (Horvath 2013) and additional DNAm based biomarkers of aging to a cohort of South African perinatally infected adolescents to investigate whether accelerated aging is associated with changes in brain structure or white matter integrity. We hypothesised that accelerated epigenetic aging and CPE score of ART would be associated with altered neuro-developmental trajectories in PHIV+ adolescents, and that this will be detectable at the macro and microstructure levels of brain anatomy.

Methods

Participants

A cross-sectional analysis of enrollment data from a neurological substudy of the Cape Town Adolescent Antiretroviral Cohort (CTAAC), a longitudinal study of PHIV+ adolescents on ART in Cape Town, South Africa was done. The CTAAC study aims to investigate markers of chronic disease processes and progression in five key areas (general adolescent development, neurocognitive function, pulmonary disease, cardiovascular function, and musculoskeletal disease) in perinatally infected adolescents on ART over a 48 month period. For the CTAAC neurological substudy, PHIV+ adolescents were recruited from seven routine ART services across Cape Town, and were eligible to participate if they were aged 9–12 years, had been on ART for longer than 6 months, and knew their HIV-status. Exclusion criteria were: 1) an uncontrolled medical condition, such as diabetes mellitus, epilepsy, or tuberculosis (TB) requiring hospital admission; 2) an identified CNS condition, including meningitis (TB or bacterial), cerebrovascular accident, lymphoma, history of head injury with loss of consciousness greater than 5 minutes or any radiological evidence of skull fracture, history of perinatal complications including hypoxic ischemic encephalopathy or neonatal jaundice requiring exchange transfusion, or neurodevelopmental disorder not attributed to HIV 3) no consent and assent obtainable. A clinical neuroradiologist examined all the MRI scans to look for evidence of other secondary causes of CNS effects (including congenital infections such as cytomegalovirus). A CNS penetration effectiveness (CPE) score was calculated for each participant based on the ARVs included in their personalised drug regimen. Each ARV is allocated a score between 1 and 4

with a higher score indicating higher penetration, see Letendre et al (2010) for a full rubric. In our sample patients CPE scores ranged from 2 to 10.

The primary caregiver of each adolescent provided written informed consent prior to participation, and child assent was obtained. The study was approved by the University of Cape Town's Faculty of Health Sciences Human Research Ethics Committee and the University of California Los Angeles Institutional Review Board. Data and samples were obtained from 204 PHIV+ CTAAC participants between 2013 and 2015. Baseline health and sociodemographic questionnaires were administered to obtain general health information, past history and data on ancestry, and treatment.

Epigenetic Characterization

Blood Collection and Processing—Blood samples were drawn at enrollment. DNA extraction from blood samples was done using the QIAsymphony DSP DNA Midi kit and protocol. DNA was quantified using BioDrop (Whitehead Scientific, South Africa) and normalized to a concentration of 5-10ng/ul. All samples were tracked using a Laboratory Information Management System (LIMS; Freezerworks, USA).

DNA Methylation—DNA methylation analysis was performed with the Illumina Infinium Methylation EPIC BeadChip, which measures bisulfite-conversion-based, single-CpG resolution DNAm levels at 866,836 CpG sites in the human genome. The standard protocol of Illumina methylation assays quantifies methylation levels by the β value using the ratio of intensities between methylated (signal A) and un-methylated (signal B) alleles. Specifically, the β value is calculated from the intensity of the methylated (M corresponding to signal A) and un-methylated (U corresponding to signal B) alleles, as the ratio of fluorescent signals $\beta = \text{Max}(M,0)/[\text{Max}(M,0) + \text{Max}(U,0) + 100]$. Thus, β values range from 0 (completely un-methylated) to 1 (completely methylated)(Dunning et al. 2008). We used the noob normalization method(Triche et al. 2013), which is implemented in the "minfi" R package(Aryee et al. 2014).

DNA methylation age and the epigenetic clock—Several highly accurate epigenetic biomarkers of chronological age have been proposed.(Garagnani et al. 2012; Horvath 2013). These biomarkers use weighted averages of methylation levels at specific CpG sites to produce estimates of age (in units of years), referred to as "DNA methylation age" (DNAm age) or "epigenetic age". Even after adjusting for chronological age, the DNAm age of blood has been found to be associated with the risk for all-cause mortality(Christiansen et al. 2015; Perna et al. 2016), frailty(Breitling et al. 2016), and cognitive functioning(Chouliaras et al. 2018).

We employed two different epigenetic age estimators . First, we used the pan tissue DNAm age estimator (Horvath (2013),(Horvath 2013) which is defined as a prediction method of age based on the DNAm levels of 353 CpGs. Second, we applied the Hannum measure of DNAm age based on 71 CpGs, which was developed using DNA methylation data from blood(Armstrong et al. 2017).

After adjusting DNAm age for chronological age, a measure of epigenetic age acceleration is derived. Here we focus on two widely used measures of epigenetic age acceleration denoted by *AgeAccelerationResidual* and Extrinsic epigenetic age acceleration *(EEAA)*, respectively. By definition, both measures are independent of chronological age (at the time of blood draw). For both measures of age acceleration, positive (negative) values indicate that the blood sample is older (younger) than expected based on chronological age. The mathematical definition of these measures is briefly reviewed here. AgeAccelerationResidual is defined as the (raw) residual resulting from regressing the pan tissue DNAm age estimate on chronological age. EEAA can be interpreted as an enhanced version of the Hannum measure of DNAm age estimator because it up-weights the contributions of age-related blood cell counts(Horvath et al. 2016).

The EEAA measure has exhibited the strongest predictive association with all-cause mortality and it revealed the strongest association with cognitive functioning measures in our previous study(Horvath et al. 2018). AgeAccelerationResidual is less confounded by changes in blood cell composition, applies to all tissue samples, is particularly accurate in adolescents, and previously used a study of HIV infected adults(Horvath 2013; Levine et al. 2016). While AgeAccelerationResidual is relatively robust with respect to changes in blood cell composition, EEAA capitalizes on age related changes in blood cell types and captures aspects of immunosenescence(Chen et al. 2016).

The epigenetic clock method and software applies to data generated using any Illumina platform (including the EPIC array). Missing CpG probes were automatically imputed by the software. Mathematical details and software tutorials for the epigenetic clock can be found in the Additional files of (Horvath 2013). An online age calculator can be found at our webpage: http://labs.genetics.ucla.edu/horvath/dnamage/.

Neuroimaging

Image acquisition—Structural and Diffusion weighted imaging was performed at the Cape Universities Brain Imaging Centre on a 3T Siemens Allegra scanner. A single-channel transmit-receive head coil was used with the following parameters: TR = 8800ms, TE = 88ms, field-of-view of 220mm, 1.8 x 1.8 x 2.0 mm^3 image resolution, 65 slices, 0% distance factor and 2x GRAPPA acceleration. Images were acquired in an axial orientation with 30 gradient directions at b = 1000mm/s^2, and 3 directions with b = 0mm/s^2. The acquisition was repeated 3 times to allow for redundancy in data. A multi-echo MPRAGE T1-weighted image was acquired with the following parameters: FOV = 256 x 256mm, TR = 2530ms, TE = 1.53/3.21/4.89/6.57ms, TI = 1100ms, flip angle = 7°, 144 slices, in-plane resolution = 1.3 x 1.0mm^2 and slice thickness of 1.0mm.

DTI Pre-processing—Diffusion weighted images were corrected for eddy current distortion within FSL 5.0.1 and imported into MATLAB R2013b for processing. This entailed the affine registration to the average b= 0mm/s^2 image of the first acquisition. For each of the acquisitions, outlier data points were determined by calculating the Z-values at the 25^{th} and 75^{th} percentile of the registered diffusion image. Any data points that were 3 SD from the mean were excluded. The corrected images were exported to FSL 5.0.1 after

correction. In FSL 5.0.1 images underwent BET to remove any non-brain tissue and fit a linear tensor model to produce fractional anisotropy (FA) and mean diffusivity (MD) and radial diffusivity (RD) maps.

Fractional anisotropy images were analysed with the TBSS pipeline(Smith et al. 2006). Each participant's FA was registered to a study-specific target. This target was determined by registering each participant to every other participant. The mean square displacement coefficient of each image was calculated and the participant with the lowest mean displacement was chosen as a representative target for the group. After registration to the study-specific target, each image was then up-sampled to MNI space, taking into account the previous transformation parameters. An average FA was created and thinned to produce a mean FA skeleton with a threshold of 0.2. This skeleton is representative of the centres of white matter tracts common to the group. Registration and skeleton projection were also applied to the MD, images as described above.

Freesurfer preprocessing—T1-weighted images were processed with Freesurfer V5.3 on the Lengau cluster at the Centre for High Performance Computing (CHPC), Rosebank, Cape Town, South Africa. The pipeline has been described previously(Fischl and Dale 2000; Fischl et al. 2001). T1-weighted images were normalized, bias-field corrected and skull-stripped. Inner and outer cortical surfaces were modeled as triangular tessellation. Cortical thickness measurements were obtained by calculating the distance (in mm) between pial and grey-white matter surfaces at each vertex location. Cortical surface area was calculated as the average of the grey matter vertices over regions. The vertex data was normalized to the "fsaverage" template included with Freesurfer by utilizing a curvature matching technique. For volumetric data, the brain was segmented into volume-based labels utilizing probabilistic methods. After reconstruction, each individual scan was checked for any major errors in segmentation, corrected and rerun if needed.

Statistical analysis

To assess the roles of chronological age, sex, CPE score and epigenetic age acceleration on brain structure in PHIV+, we developed backward elimination stepwise multiple regression models in R (version 3.4.3, 2017) covering cortical surface area, cortical thickness, and volume, as well as DTI (FA, MD, RD, and AD) values. A correlation of all the variables was done as part of standard assumption testing, in order to assess independence of the variables. The variables fit the required assumptions. Each brain measure was modelled as dependent variable of a multivariable regression model. The backward feature elimination procedure started out with the full regression model that included all predictors (including chronological age, sex, CPE, and epigenetic age acceleration). This was done in order to assess which variables were most associated with brain structural values, leaving only those variables in the model that contributed to the anatomical variance. EEAA and AgeAccelerationResidual were tested separately to avoid multi-collinearity in the regression models. The proposed sample size of 200 HIV infected adolescents in the CTAAC neurological substudy was based on the correlation of imaging findings (e.g. white matter fiber bundles in the frontal brain region) with tests of cognitive function (eg, executive function); the proposed sample size would allow >90% power to detect correlations of >0.2

Results

Demographics

The CTAAC neurological substudy enrolled 204 PHIV+. 180 PHIV+ were included in the final analysis, as 24 participants were excluded due to movement causing poor image quality. The mean CPE score of the PHIV+ group was 7.9. Their mean DNAm age (16.01 years) was higher than their mean chronological age (10.77 years). The mean CD4 cell count of the group was 961.8, median vL 0 copies/mL, 85% of the cohort was virally suppressed with a vL of <50 copies/mL.

Regression modelling

In each case, sex, chronological age, CPE, AgeAccelerationResidual or EEAA, were modelled as predictor variables with the value of the regional brain characteristic (i.e. thickness, Area, Volume, FA, or MD) as the dependent (outcome) variable. Each brain region and characteristic led to a separate regression model. Given the stepwise approach (backward feature elimination) taken in the multiple regression analyses, all predictors that had a predictive value of $p<0.15$ were left in the model and thus contribute to the final R^2 value, however, only those that had significant predictive value (nominal $p<0.05$) are in bold typeface in the results tables (Tables 2 and 3). The inclusion of each predictor variable is indicated by its beta value in the relevant column of the results tables. The R^2 values indicate the percentage of the brain characteristic variance explained by each model.

The results of the regression analyses in the PHIV+ showed many brain regions had altered structural characteristics associated with AgeAccelerationResidual or EEAA. AgeAccelerationResidual was associated with alterations in cortical thickness (pars opercularis and precentral gyrus), surface area (multiple regions), volume (amygdala and total grey matter) and microstructural FA values in the external capsule (Table 2). EEAA was also associated with many regional structural changes in cortical thickness (cuneus and orbitofrontal), surface area (multiple regions), volume (amygdala and mid anterior CC) and microstructural changes in FA (external capsule and superior cerebellar peduncle), MD (internal capsule and cingulum), RD (internal capsule and sup fronto occipital fasciculus) and AD (internal capsule, cingulum, sup fronto occipital fasiculus) (Table 3).

With regards to the macrostructural ROIs, sex was both negatively and positively associated with area and volume across a number of brain regions indicating both lower and higher values in females. As individual predictor variables, the epigenetic aging markers (AgeAccelerationResidual and EEAA) contributed more to the brain macrostructural values than chronological age. CPE positively contributed to area of the inferior temporal gyrus, and volume of the brain stem. With regards to the white matter microstructural regions, chronologocal age was not a significant contributor to any of the DTI ROIs. Sex only contributed to one FA region in the retrolenticular internal capsule with girls having a higher value, and lower MD and AD of the superior cerebellar peduncle. CPE was negatively associated with MD and AD in the superior corona radiata and fronto-occipital fasciculus when modelled with AgeAccelerationResidual, and when modelled with EEAA. See Table 2 for full results of AgeAccelerationResidual and Table 3 for full results of the EEAA models.

Discussion

This is the first study to show that epigenetic age acceleration in PHIV+ adolescents is associated with alterations in brain morphology. Specifically, epigenetic age acceleration and extrinsic epigenetic age acceleration (EEAA) were associated with multiple alterations in cortical thickness, volume and cortical surface area. In addition white matter microstructural integrity of the superior cerebellar peduncle, internal capsule, superior fronto-occipital fasiculus, external capsule and cingulum was associated with epigenetic age acceleration and EEAA in PHIV+ adolescents. DTI and MRI studies have examined brain macro and microstructure in children living with HIV compared to an uninfected control group. In children with HIV, even when long-term clinically and virologically controlled, lower brain volumes and poorer white matter integrity have been reported compared to matched controls (Hoare et al. 2012; Buch et al. 2016; Cohen et al. 2016). HIV infected children have white matter abnormalities measured by FA, despite early ART, suggesting that ART does not fully protect the white matter from either peripartum or in utero infection(Ackermann et al. 2016). The current exploratory results suggest that HIV may work via epigenetic mechanisms to contribute to brain structure. EEAA in PHIV+ was associated with increases in MD and RD, higher MD and RD are traditionally representative of poorer directional diffusion and inflammation(Song et al. 2002). The interaction with brain macrostructure mostly yielded increases in brain volumes and area. While in most cases, larger or thicker neuroimaging derived measures are considered to be associated with healthier brains, this is not always the case. Randall et al. investigated abnormalities in subcortical grey matter volumes in PHIV+ and HIV- 5yr old Xhosa children who were initiated to ART before 18 months of age. The group reported that PHIV+ children had larger subcortical volumes compared to the uninfected group and that this difference was largely driven by children initiated to ART after 12 weeks of age(Randall et al. 2017). The authors suggest that larger differences in the later-initiated group was evidence of neuroprotective effects of earlier treatment. Though speculative, it is possible that our observed positive correlation between epigenetic age acceleration/HIV and volume reflects ongoing disease mechanisms among individuals with accelerated aging; but further studies directly assessing markers of inflammation are needed to test this hypothesis. Further, CD4+ T cell counts were negatively correlated with epigenetic age acceleration in PHIV+ adolescents as previously reported by our group.

Little research is available on the interaction of epigenetic age acceleration and brain macro and microstructure in children or adolescents. Epigenetic mechanisms such as DNAm act as an interface between the environment and the genome, and could therefore play a role in the development of brain structures. Evidence suggests that the dynamic regulation of gene expression through epigenetic mechanisms is at the interface between environmental stimuli and long lasting molecular, cellular and complex behavioral phenotypes acquired during periods of developmental plasticity(Fagiolini et al. 2009). A positive effect of early supportive parenting on healthy hippocampal development, a brain region key to memory and stress modulation, has previously been documented in school children(Luby et al. 2012). Adolescents who grew up in poor economic conditions have higher baseline glucocorticoid levels, as do adolescents whose mothers were depressed in the early postnatal period(Lupien et al. 2009). Alterations in grey matter volume and the neuronal integrity of

the frontal cortex, and reduced size of the anterior cingulate cortex, have been reported in adolescents exposed to early adversity(Cohen et al. 2006). The relevance of these findings is underscored by our recent findings in the same PHIV+ participants that EEAA remained negatively associated with executive functioning, working memory and processing speed in our cohort, even after adjusting for HIV status, educational level (assessed by highest grade, and repeated grade), ethnicity, and household income(Horvath et al. 2018). That is, the accelerated aging associated with HIV and the resulting gradual neurologic changes that occur have as their endpoint measurable neurocognitive deficits, which in turn translate into poorer scholastic functioning. Importantly, our findings provide further evidence that blood is a promising surrogate for brain tissue when studying the effects of HIV on neurocognitive functioning(Horvath 2013).

Although a fair amount of research has focused on the potential improved neurocognitive outcomes of using ART with high CPE in adults, very little is understood about ART CPE in adolescents living with HIV(Smith and Wilkins 2014). This study found CPE scores to positively contribute to volume of the brain stem and inf temporal gyrus. CPE was negatively associated with MD and AD in the superior corona radiata and fronto-occipital fasciculus when modelled with AgeAccelerationResidual, and when modelled with EEAA. These findings suggest that a higher CPE scores may be associated with higher brain stem volumes and reduced microstructural inflammation of regional white matter. The association of CPE scores with neurocognitive outcomes in school-aged PHIV+ children have been mixed. CPE scores have showed no association with neurocognitive outcomes(Crowell et al. 2015; Lazarus et al. 2015) in some studies while in others participants on ART with high baseline CPE scores had verbal IQ scores that were significantly higher than in those on regimens with low CPE scores(Kapetanovic et al. 2012).

Several limitations of this study should be emphasized. First, the cross-sectional design limits causal inferences. Longitudinal follow-up is underway to better understand the impact of epigenetic age acceleration on brain structure and functioning in later adolescence. Although other congenital infections and incidental CNS abnormalities were excluded as far as possible on history, clinical examination and on clinical review of the MRI scans, it remains a possibility that there may be some overlapping effects of undiagnosed conditions such as congenital cytomegalovirus. Future research should focus on delineating specific factors that may account for accelerated aging in PHIV+ youth.

Conclusion

This is the first cohort study to examine the relationship between epigenetic age acceleration in blood and various measures of brain structure in adolescents living with HIV. The study took place in South Africa, one of the countries most affected by HIV/AIDS with the highest rate of new HIV infections in the world. Longitudinal follow-up of our cohort will be crucial for determining the impact of epigenetic aging in adolescents living with HIV. Correlates of epigenetic aging need to be elucidated, including associations with measures of viral load, treatment duration, concurrent infections, and ART prescription and treatment adherence in order to understand their impact on the long-term development of neurocognitive disorders in pediatric HIV.

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Table 1:

Demographic and clinical characteristics

Variable	PHIV+ N=180
Age in years: Mean (SD)	10.77(0.87)
DNAm age: Mean (SD)	16.01(3.54)
Gender: Male/Female	85/95
Ethnicity: Black African/Other	167/13
Home Language: isiXhosa/Other	165/15
Low household income: (%) (<R2500 per month)	98.5%
Viral load: Median (IQR) (copies/mL)	0(40)
CD4 count: Mean(SD)	961.8(492.4)

J Neurovirol. Author manuscript; available in PMC 2023 April 01.

Table 2:

Results of Age AccelerationResidual and additional predictors on regional brain characteristics in PHIV

Sample	Structural measure	Brain region	Hem.	Sex β(p)	Age β(p)	CPE β(p)	Age acceleration β(p)	R^2	P
HIV infected									
	Thickness	Pars opercularis	R	**.14(.047)**		−.14(.060)	**.16(.031)**	.069	.006
		Insula	L	**.15(.044)**		**−.15(.046)**		.042	.022
		Precentral gyrus	L	**.171(.022)**			**.15(.049)**	.046	.016
	Area	Inf temporal gyrus	L	**−.24(.001)**		.14(.052)	**.20(.007)**	.115	<.001
		Sup temporal gyrus	L	**−.16(.033)**			**.20(.008)**	.135	<.001
		Inf temporal gyrus	R	−.14(.053)		**.15(.040)**	**.09(.013)**	.071	.005
		Lateral occipital gyrus	L	**−.27(.000)**		.14(.060)	**.15(.043)**	.110	<.001
		Lat orbitofrontal gyrus	L	**−.24(.001)**			**.17(.017)**	.094	<.001
		Transverse temporal gyrus	R	**−.25(.001)**		.14(.065)	**.17(.020)**	.105	<.001
		Caudal middle frontal	L	**−.25(.000)**			**.26(.000)**	.140	<.001
		Caudal anterior cingulate	L	−.15(.052)			.14(.067)	.043	.020
		Paracentral gyrus	L	**−.32(<.001)**			**.17(.018)**	.142	<.001
		Pars orbitalis	L	**−.27(<.001)**			**.29(<.001)**	.172	<.001
		Posterior cingulate	L	**−.25(<.001)**			**.20(.005)**	.116	<.001
		Precentral gyrus	L	**−.29(<.001)**			**.22(.002)**	.144	<.001
		Rostral anterior cingulate	L	**−.24(.001)**			**.16(.024)**	.093	<.001
		Rostral middle frontal gyrus	L	**−.23(.001)**			**.27(<.001)**	.135	<.001
		White matter surface area	L	**−.26(.001)**			**.18(.011)**	.111	<.001
		Caudal middle frontal	R	**−.23(.002)**			**.17(.020)**	.088	<.001
		Inf parietal gyrus	R	**−.25(.001)**			**.17(.017)**	.012	<.001
		Paracentral	R	**−.24(.001)**			**.24(<.001)**	.126	<.001
		Pars orbitalis	R				**.28(.001)**	.080	<.001
		Pars triangularis	R	**−.21(.003)**			**.25(.001)**	.116	<.001
		Precentral	R	**−.20(.007)**			**.21(.004)**	.091	<.001
		Rostral middle frontal	R	**−.16(.027)**			**.30(<.001)**	.123	<.001
		Sup frontal	R	**−.22(.002)**			**.21(.004)**	.102	<.001
		Insula	R	**−.29(<.001)**			**.16(.027)**	.120	<.001

J Neurovirol. Author manuscript; available in PMC 2023 April 01.

Sample	Structural measure	Brain region	Hem.	Sex β(p)	Age β(p)	CPE β(p)	Age acceleration β(p)	R^2	P
		White matter surface area	R	**−.19(.008)**			**.19(.010)**	.081	<.001
	Volume	Amygdala	R	**−.22(.003)**			**.15(.038)**	.076	.001
		Total gray matter volume	L+R	**−.22(.003)**			**.14(.049)**	.077	<.001
		Brain stem	n/a	**−.15(.040)**	**−.20(.006)**				
	FA	Retrolenticular part of the internal capsule	L	**.18(.043)**			.17(.058)	.071	.030
		External capsule	R				**.23(.009)**	.055	.009
		External capsule	L				**.22(.012)**	.050	.012
	MD	Superior Corona radiata	R			**−.21(.019)**		.044	.019
		Sup fronto occipital fasciculus	L			**−.35(<.001)**		.126	<.001
		Sup cerebellar peduncle	R	**−.19(.033)**				.037	.033
	AD	Sup cerebellar peduncle	R	**−.24(.008)**				.056	.008
		Sup corona radiata	R			**−.21(.022)**		.042	.022

Standardized beta scores (β) for each predictor variable in each model are shown in the relevant named columns, with p values in parentheses. R^2 values and p values in the final column are for each full model. Each row is one full model. Included covariates that are significant predictors (p<0.05) in each model are highlighted in bold.

Table 3:

Results of EEAA and additional predictors on regional brain characteristics in PHIV

Sample	Structural measure	Brain region	Hem.	Sex β(p)	Age β(p)	CPE β(p)	EEAA β(p)	R^2	P
HIV infected									
	Thickness	Cuneus	L				**−.17(.024)**	.065	.003
		Insula	L	**.15(.044)**		**−.15(.046)**		.042	.022
		Cuneus	R		**−.18(.017)**		**−.16(.037)**	.056	.007
		Med orbitofrontal	R	**.21(.006)**			**−.15(.040)**	.062	.004
	Area	Caudal middle frontal	L	−.14(.061)			**.18(.016)**	.099	<.001
		Rostral middle frontal	L	**−.26(<.001)**			**.20(.005)**	.104	<.001
		Pars orbitalis	L	**−.31(<.001)**			**.18(.011)**	.123	<.001
		Rostral anterior cingulate	L	**−.27(<.001)**			**.21(.004)**	.109	<.001
		Inf parietal	R	**−.28(<.001)**			**.15(.035)**	.095	<.001
		Med orbitofrontal	R	−.14(.052)			**.19(.013)**	.052	.009
		Paracentral	R	**−.27(<.001)**			**.15(.041)**	.090	<.001
		Precentral	R	**−.22(.003)**			**.16(.035)**	.070	.002
		Rostral middle frontal	R	**−.19(.008)**			**.20(.008)**	.072	.001
		Sup frontal	R	**−.25(.001)**			**.20(.006)**	.099	<.001
		Transverse temporal	R	**−.27(<.001)**		.14(.058)	**.16(.034)**	.100	<.001
	Volume	Amygdala	R	**−.24(.001)**			**.17(.020)**	.082	<.001
		Brian stem	n/a	**−.27(<.001)**		**.17(.020)**		.099	<.001
		Mid anterior CC	n/a	**−.19(.009)**			**.16(.032)**	.060	.005
	FA	Sup cerebellar peduncle	L				**.19(.031)**	.038	.031
		External capsule	R				**.20(.026)**	.040	.026
		Corticospinal tract	L	**−.18(.046)**	.16(.080)			.055	.033
	MD	Retrolenticular internal capsule	L	−.13(.138)			**.20(.024)**	.057	.029
		Cingulum	L				**.19(.038)**	.035	.038
		Sup fronto occipital fasciculus	L			**−.35(<.001)**		.126	<.001
	RD	Retrolenticular internal capsule	L				**.16(.083)**	.024	.083
		Sup fronto occipital fasciculus	L				**.30(.001)**	.089	<.001
	AD	Retrolenticular internal capsule	L		−.17(.057)		**.22(.014)**	.074	.010

Sample	Structural measure	Brain region	Hem.	Sex β(p)	Age β(p)	CPE β(p)	EEAA β(p)	R^2	P
		Sup corona radiata	R			**−.21(.022)**		.042	.022
		Cingulum	L				**.17(.066)**	.027	.066
		Sup fronto occipital fasciculus	R				**.19(.035)**	.036	.035
		Sup fronto occipital fasciculus	L				**.20(.025)**	.041	.025

Standardized beta scores (β) for each predictor variable in each model are shown in the relevant named columns, with p values in parentheses. R^2 values and p values in the final column are for each full model. Each row is one full model. Included covariates that are significant predictors (p<0.05) in each model are highlighted in bold