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Brain Differences in Adolescents Living With Perinatally Acquired HIV Compared With Adoption Status Matched Controls

A Cross-sectional Study

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Abstract

Background and Objectives

Despite effective combination antiretroviral therapy (cART), adolescents with perinatally acquired HIV (PHIV) exhibit cognitive impairment, of which structural changes could be the underlying pathophysiologic mechanism. Prior MRI studies found lower brain volumes, higher white matter (WM) hyperintensity (WMH) volume, lower WM integrity, and differences in cerebral blood flow (CBF). However, these findings may be confounded by adoption status, as a large portion of adolescents with PHIV have been adopted. Adoption has been associated with malnutrition and neglect, which, in turn, may have affected brain development. We investigated the long-term effects of PHIV on the brain, while minimizing the confounding effect of adoption status.

Methods

We determined whole-brain gray matter (GM) and WM volume with 3D T1-weighted scans; total WMH volume with fluid-attenuated inversion recovery; CBF in the following regions of interest (ROIs): WM, GM, and subcortical GM with arterial spin labeling; and whole-brain WM microstructural markers: fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) with diffusion tensor imaging in cART-treated adolescents with PHIV visiting our outpatient clinic in Amsterdam and controls matched for age, sex, ethnic origin, socioeconomic status, and adoption status. We assessed differences in neuroimaging parameters between adolescents with PHIV and controls using linear regression models adjusted for age and sex and applied multiple comparison correction.

Results

Thirty-five adolescents with PHIV and 38 controls were included with a median age of 14.9 (interquartile range [IQR]: 10.7–18.5) and 15.6 (IQR: 11.1–17.6) years, respectively, with a similar rate of adoption. We found a lower overall FA (beta = −0.012; $p < 0.014$, −2.4%), a higher MD (beta = 0.014, $p = 0.014$, 1.3%), and a higher RD (beta = 0.02, $p = 0.014$, 3.3%) in adolescents with PHIV vs adoption-matched controls, but no differences in AD. We found comparable GM, WM, and WMH volume and CBF in ROIs between adolescents with PHIV and controls. We did not find an association between cognitive profiles and WM microstructural markers in adolescents with PHIV.

Discussion

Irrespective of adoption status, adolescents with PHIV exhibited subtle lower WM integrity. Our findings may point toward early-acquired WM microstructural alterations associated with HIV.

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Glossary

AD = axial diffusivity; **ASL** = arterial spin labeling; **cART** = combination antiretroviral therapy; **CBF** = cerebral blood flow; **DTI** = diffusion tensor imaging; **FA** = fractional anisotropy; **FLAIR** = fluid-attenuated inversion recovery; **FOV** = field of view; **FSL** = FMRIB Software Library; **GM** = gray matter; **MD** = mean diffusivity; **NOVICE** = neurologic, cognitive and visual performance in perinatally HIV infected children; **PHIV** = perinatally acquired HIV; **RD** = radial diffusivity; **ROI** = region of interest; **TE** = echo time; **TR** = repetition time; **WM** = white matter; **WMH** = WM hyperintensity.

HIV is known for its neurotropic properties.[1] Despite effective combination antiretroviral therapy (cART), children and adolescents with perinatally acquired HIV (PHIV) still experience neurologic complications.[2] These findings suggest structural brain changes or brain damage associated with HIV. The pathophysiologic mechanisms underlying this cerebral damage are yet to be fully elucidated; however, it is hypothesized to include multifactorial effects, such as lasting effects of damage acquired during untreated perinatal HIV infection, HIV-associated neuroinflammation, and toxicity associated with cART.[3] The introduction of cART led to a substantial decrease in the incidence of severe neurologic complications such as HIV-associated encephalopathy.[4] Yet evidence suggests that despite cART, adolescents with PHIV still exhibit a higher prevalence of cognitive impairment compared with uninfected peers, including lower IQ and poorer executive function.[5,6]

MRI biomarkers are sensitive in detecting early HIV-related brain changes.[7] For example, a large study found lower gray matter (GM) and white matter (WM) volume in children with PHIV compared with healthy controls.[8] In addition, WM damage can be investigated by determining WM hyperintensities (WMHs) typically assessed using the fluid-attenuated inversion recovery (FLAIR) MRI sequence. Current evidence concerning WMH volume in children with PHIV on cART is scarce and contrasting: 1 study reported a higher volume of WMH, whereas most studies found no differences compared with controls in volume or presence.[9-11] In adults, evidence suggests that a positive HIV status is associated with a higher WMH volume compared with healthy adults,[12] which, in turn, is associated with cognitive impairment.[13] To further examine WM microstructure, diffusion tensor imaging (DTI)—which assesses the diffusion of water in the WM—can be used. In children and adolescents with PHIV, both longitudinal and cross-sectional studies report (persistent) lower whole-brain fractional anisotropy (FA) compared with controls, suggesting lower WM integrity.[9,10,14]

Because WMHs are presumed to be part of the small vessel disease spectrum,[15] it is of interest to assess cerebral perfusion as well. This can be investigated noninvasively using arterial spin labeling (ASL) MRI, which measures cerebral blood flow (CBF) using arterial blood water as an endogenous tracer. Despite initially higher CBF in the putamen and caudate nucleus in adolescents with PHIV compared with matched healthy controls, we reported comparable CBF development over time in adolescents with PHIV and matched controls.[16]

Although evidence suggests that children and adolescents with PHIV manifest subtle brain alterations compared with healthy controls, these studies did not match for, or report differences in, early-life characteristics, such as nutrition history or adoption status.[8-10,14,16] These factors are important because brain maturation is at a critical stage in early life[17] and adoption has been associated with malnutrition and neglect,[18] which, in turn, have been associated with altered brain development.[19,20] In the Netherlands, the majority of adolescents with PHIV have been adopted.[21] While minimizing the confounding effect of adoption status–and associated early-life characteristics–we compared brain alterations in adolescents with PHIV to HIV-negative status controls, matched for age, sex, ethnic origin, socioeconomic, and adoption status. We hypothesized that, even when controlling for early-life characteristics associated with adoption status, adolescents with PHIV will still have more brain alterations compared with controls, related to their HIV status.

Methods

Study Participants

We used anonymized data from participants who enrolled in the neurological, cognitive and visual performance in perinatally HIV infected children (NOVICE) study between February 2017 and July 2018. The NOVICE study is a cohort study investigating the neurologic, cognitive, and ophthalmologic outcomes of adolescents with PHIV compared with controls, who were frequency matched for age, sex, ethnic origin, socioeconomic status, and additionally for international adoption status.[22] Adolescents with PHIV visiting the outpatient clinic of the Emma Children's Hospital (Amsterdam) or previously participated in our study were approached for participation. This outpatient clinic is 1 of 4 Dutch pediatric HIV centers that adolescents with PHIV visit biannually for a checkup. Internationally adopted HIV-negative controls were recruited through 2 government licensed organizations, for these organizations arrange adoptions from the sub-Saharan region. The following exclusion criteria were used: current or past neurologic or psychiatric disorders not associated with HIV, a history of traumatic brain injury resulting in loss of consciousness of more than 30 minutes, intracerebral neoplasms, and MRI contraindications including metal implants or claustrophobia; the complete criteria for noninclusion have been previously published in detail.[9]

Standard Protocol Approvals, Registrations, and Patient Consents

The Institutional Review Board of the Academic Medical Center, part of the Amsterdam University Medical Center, approved the study with registration number: NL58216.018.016 on October 21, 2016. The NOVICE study is registered at the Dutch Trial Registry (registration number: NL6813). We adhered to the Declaration of Helsinki and obtained written informed consent from all participants older than 12 years and from participants' parents younger than 18 years.

MRI Data Acquisition and Image Processing

We performed an MRI scan of the brain of all participants using a 3T MRI scanner (Ingenia, Philips, Best, The Netherlands) equipped with a 16-channel phased-array head coil.

3D T1 Weighted

We measured brain volumes using sagittal 3D T1-weighted ($T1_w$) scans with a magnetization-prepared rapid gradient-echo sequence with the following scanning parameters: echo time (TE) = 3.18 ms, repetition time (TR) = 7.0 ms, field of view (FOV) = 256 × 240 × 180 mm^3, flip angle = 9°, and isotropic voxel size = 1 mm^3. Computational Anatomy Toolbox 12 was used to segment the GM, WM, and CSF,[23] implemented within the ExploreASL (version 1.2.0) toolbox.[24]

3D FLAIR

Sagittal 3D FLAIR scans were obtained using the following parameters: TR/TE = 4,800/356 ms, TI = 1,650 ms, FOV = 250 mm × 250 × 180 mm^3, and voxel size = 1.1 × 1.1 × 0.56 mm^3. To determine the presence of any WMH, 1 investigator (J.v.G.) reviewed and manually segmented all FLAIR scans with supervision of an experienced neuroradiologist (L.R.) using segmentation software ITK-SNAP version 3.4.0 (Philadelphia, PA, and Salt Lake City, UT).[25] ITK-SNAP computed the total WMH volume after manual segmentation. We excluded scans in which WMH could not be identified because of severe head motion, as defined by the neuroradiologist. To determine the intrarater reliability, 20 FLAIR scans were randomly selected and rated twice. Intrarater reliability was assessed using percentual agreement and Cohen weighted κ coefficient.

ASL

CBF maps were obtained using 2D echoplanar imaging pseudo-continuous ASL with TE/TR 16/4,000 ms, FOV = 240 × 240 mm^2, voxel size 3 × 3 × 6.6 mm^3, 20 axial 6 mm slices with 0.6 mm slice gap, labeling duration = 1,650 ms, postlabeling delay = 1,525–2,230 ms, 30 control and label pairs. We used ExploreASL (version 1.2.0) to process ASL images. We chose the following regions of interest (ROIs): cortical GM, WM, and subcortical GM regions: caudate nucleus, putamen, and thalamus, because of cerebral injury in children with PHIV mostly occurring in the GM, WM, and subcortical regions and because of the association between subcortical brain volume and cognitive impairment.[4,26] There were no statistically significant hematocrit differences, adjusted for age and sex, between adolescents with PHIV and controls (p = 0.910); hence, we assumed the same blood T1 values for quantification in both groups.[27] Because of a scanner software upgrade, we used individual CBF ratios to allow methodological correct comparison, in line with our previous publication on CBF in adolescents with PHIV.[16] We calculated CBF ratios of ROIs by dividing the CBF value of an ROI (in mL/100 g/min) by the mean GM CBF of controls (in mL/100 g/min). We excluded scans in case of insufficient quality due to head motion or artifacts, which was done in agreement between 2 investigators (J.v.G. and H.J.M.M.M.).

DTI

In line with our previous analyses, we assessed WM microstructure using the following whole-brain parameters: FA, mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AD). We used the following acquisition sequence: spin-echo single-shot echoplanar imaging along 46 directions with b = 1,000 s/mm^2 and 4 averages with b = 0 s/mm^2; TE/TR = 92/9,476 ms, FOV = 224 × 224 mm^2, 70 slices, and an isotropic voxel size of 2.0 mm^3. We used FMRIB Software Library (FSL) 4.1.6 (University of Oxford, Oxford, UK) to analyze the DTI scans.[28,29] We denoised the data and corrected for eddy currents using MRtrix3 and Eddy (FSL), respectively.[30] We replaced outliers defined by head motion equal or more than 3 SDs by Gaussian Process predicted values.[31] Diffusion-weighted volumes (b > 0) were rigid-body registered to the non–diffusion-weighted volume (b = 0). Next, Gibbs artifacts were corrected using an in-house Matlab script. We used FSL's DTIFIT to fit the tensors to the diffusion data to create FA, MD, AD, and RD maps. These maps were analyzed using FSL's Tract-Based Spatial Statistics,[28,29] using the most representative study-specific FA image as the target image.[32] A mean FA skeleton, which represented the center of all study-specific common WM tracts, with a threshold of p > 0.20 was created after resampling into the common space. To create skeletonized data, FA images of all participants were projected onto the mean FA skeleton. Whole-brain FA, MD, AD, and RD values were calculated by averaging the entire WM skeleton for each subject.

Demographic and HIV-Related Variables

The following (historical) cART- and HIV-related characteristics of the participants with PHIV were collected from patient records or provided by the Dutch HIV monitoring foundation: peak HIV viral load (zenith), $CD4^+$ T-cell nadir, cART use, duration of treatment, and HIV historical classification. We defined cART as the use of at least 3 antiretroviral drugs from a minimum of 2 drug classes. In all controls, we confirmed HIV-negative status as reported previously.[22]

Cognitive Function

A neuropsychologist performed neuropsychological assessments using a test battery adapted to the Dutch language to assess various cognitive domains including IQ, processing speed, learning ability, visual-motor function, and executive function. For IQ and processing speed the Wechsler Intelligence Scale for Children and Wechsler Adult Intelligence Scale were used. The Rey Auditory Verbal Learning Test was used to assess learning ability. The Beery-Buktenica

Table 1 Participants' Characteristics at Enrollment

	n	PHIV (n = 35)	n	Controls (n = 38)	*p* Value
Age (y)	35	14.9 (10.7–18.5)	38	15.6 (11.1–17.6)	0.855[Y]
Female sex	35	18 (51%)	38	22 (58%)	0.642[Z]
Ethnic origin					0.102[Z]
Black	35	30 (86%)	38	29 (76%)	
Other		5 (14%)		9 (24%)	
International adoption	35	16 (46%)	38	12 (32%)	0.238[Z]
Age at adoption (y)	16	3.3 (2.5–4.8)	12	2.9 (1.2–5.2)	0.745[Y]
Height (m)	33	1.59 (1.46–1.68)	38	1.63 (1.52–1.73)	0.450[Y]
Weight (kg)	33	50 (36–64)	38	56 (41–67)	0.284[Y]
BMI (kg/m^2)	33	19.0 (17.6–21.9)	38	20.2 (18.5–23.4)	0.131[Y]
Hematocrit (L/L)	34	0.40 (0.39–0.45)	38	0.41 (0.39–0.43)	0.910[Y]
Age at HIV diagnosis (y)	35	2.1 (0.6–3.8)	—	—	—
CDC HIV category					
NA		20 (57%)	—	—	—
B		9 (26%)	—	—	—
C		6 (17%)	—	—	—
Nadir CD4$^+$ T-cell *Z* score	33	−0.85 (0.56)	—	—	—
Zenith HIV viral load (^{10}log)	32	5.3 (4.7–5.7)	—	—	—
Age at cART initiation	32	2.8 (1.1–5.7)	—	—	—
Duration of cART	32	9.0 (7.0–15.3)	—	—	—
Undetectable viral load at assessment	35	33 (94%)	—	—	—
MRI of good quality for assessment[a]					
$T1_w$	35	30 (86%)	38	29 (76%)	0.380[Z]
DTI	35	27 (77%)	38	29 (76%)	0.999[Z]
FLAIR	35	31 (89%)	38	28 (74%)	0.141[Z]
ASL	35	30 (86%)	38	29 (76%)	0.380[Z]

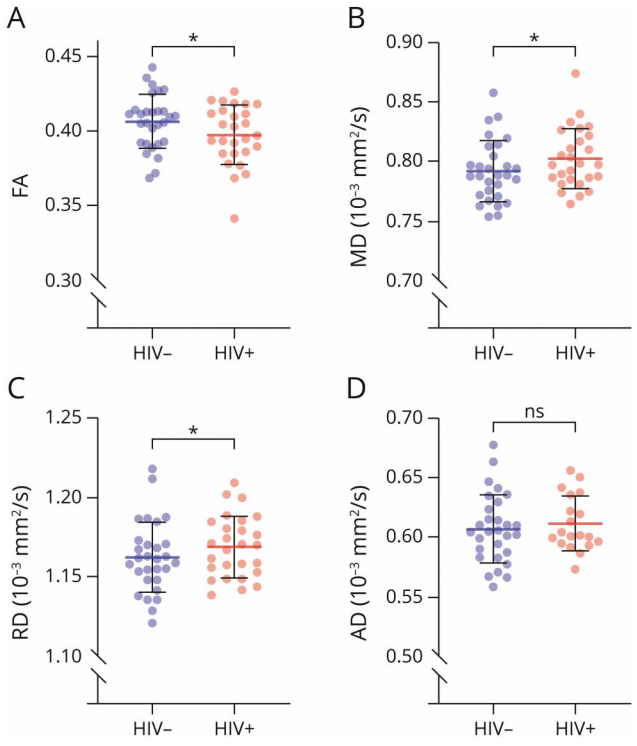
Abbreviations: ASL = arterial spin labeling; BMI = body mass index; cART = combination antiretroviral therapy; CDC = Centers for Disease Control and Prevention, DTI = diffusion tensor imaging; FLAIR = fluid-attenuated inversion recovery; NA = no or minimal symptoms; B = moderate symptoms; C = severe symptoms or (brain) AIDS.
Values noted are either the median (with interquartile range) or number (with percentage).
Y = Mann-Whitney *U* test; Z = Fisher exact test.
[a] According to the criteria outlined in the Methods.

Developmental Test of Visual-Motor Integration was used to assess visual-motor function. The Trail Making Test was used to assess executive function.[22]

Statistical Analyses

Statistical analyses were performed using R version 3.5.1 (R Core team, Vienna, Austria),[33] and a value of $p < 0.05$ was considered statistically significant. We compared demographic variables between adolescents with PHIV and controls using the Mann-Whitney *U* test (non-normally distributed) or Student *t* test (normally distributed) for continuous data and the Fisher exact test for categorical data. We assessed differences in whole-brain GM and WM volume, WM microstructural differences: FA, MD, AD, and RD, WMH volume, and CBF in the GM, WM, caudate nucleus, putamen, and thalamus between adolescents with PHIV and controls using linear regression models adjusted for age and sex. We also assessed these differences between adopted and nonadopted participants using linear regression models adjusted for age, sex, and HIV status. We additionally adjusted the model for WMH volume for WM volume. We assessed differences in IQ, processing speed, learning ability, visual motor function, and executive function between

Figure 1 Scatter Dot Plots Display WM Integrity Variables for Adolescents With PHIV (Blue) and HIV-Negative Matched Controls (Red)



(A) Fractional anisotropy. (B) Mean diffusivity. (C) Radial diffusivity. (D) Axial diffusivity. An asterisk (*) indicates a significant difference, ns = not significant. Unit for MD, RD, and AD = mm^2/s. The colored horizontal line indicates the mean value, and the black error bars indicate ± 1 SD. AD = axial diffusivity; FA = fractional anisotropy; MD = mean diffusivity; PHIV = perinatally acquired HIV; RD = radial diffusivity; WM = white matter.

adolescents with PHIV and controls using linear regression models, while adjusting for age, sex, and IQ (the latter was added in the model for all domains except IQ). In adolescents with PHIV, we assessed associations between MRI parameters and the following HIV-related characteristics: peak HIV viral load (zenith), $CD4^+$ T-cell nadir, cART use, duration of treatment, and HIV historical classification, as well as cognitive profiles. To control for multiple comparison, we applied Benjamini-Hochberg adjustments,[34] using the *p.adjust* function in R. We calculated the effect size magnitude using Cohen d with R package *effsize* and considered the following thresholds: |d| (0.01) = very small, |d| (0.2) = small, |d| (0.5) = medium, and |d| (0.8) = large.[35]

Data Availability

Anonymized data not published within this article will be made available by request from any qualified investigator.

Results

We approached 53 adolescents with PHIV and 52 controls. We included 35 adolescents with PHIV and 38 matched HIV-negative status controls with a median age at enrollment of 14.9 (IQR: 10.7–18.5) and 15.6 (IQR: 11.1–17.6) years, respectively. Reasons for noninclusion were inability to reach or no interest in participation (13 PHIV and 14 controls); 2 PHIV were excluded based on the criteria and 3 PHIV relocated. In both groups, we had similar numbers of MRIs with good quality for assessment. There were no differences in baseline characteristics between adolescents with PHIV and matched controls at enrollment (Table 1). Twenty-one adolescents with PHIV and 23 controls were previously enrolled in another cross-sectional study conducted between 2012 and 2014.[10] The data presented here were obtained in a separate study 4–5 years later between 2017 and 2018, so there is no overlap in the data presented here with our previous study.

MRI Parameters and Cognitive Profiles in Adolescents With PHIV

Adolescents with PHIV had a significantly lower FA and a higher MD and RD compared with matched controls with a medium effect size (Figure 1 and Table 2). We found no differences in GM, WM, and WMH volume between adolescents with PHIV and matched controls. There were no differences in WMH count between groups (eTable 4, links.lww.com/WNL/C221). Intrarater reliability of WMH segmentation was 90% with a Cohen weighted κ of 0.83. CBF in all ROIs was also comparable between groups. We additionally found no differences in all MRI parameters between adopted and nonadopted participants (eTable 2, links.lww.com/WNL/C221). Adolescents with PHIV had a significantly lower IQ (81 vs 92, $p < 0.001$) compared with controls (eTable 1, links.lww.com/WNL/C221).

Factors Associated With MRI Parameters

We found no associations between WM microstructural markers and historical HIV-associated characteristics (Table 3). We found that a higher age at cART initiation was associated with a higher WMH volume, and a longer duration of cART was associated with a lower WMH volume (eTable 3A, links.lww.com/WNL/C221).

No significant associations were found between other MRI parameters and HIV characteristics (eTable 3A and 3B, links.lww.com/WNL/C221). We also did not find an association between IQ and other cognitive profiles and WM microstructural markers in adolescents with PHIV (Table 3 and eTable 5, links.lww.com/WNL/C221).

Discussion

In this study, we investigated brain MRI biomarker differences between adolescents with PHIV and controls, while controlling for adoption status. We found that adolescents with PHIV had a lower FA and a higher MD and RD than controls, suggesting lower WM integrity in adolescents with PHIV, irrespective of adoption status. In contrast, we did not find group differences in GM, WM, and WMH volume or in CBF.

Our diffusion MRI findings suggest lower WM integrity in adolescents with PHIV and are in line with previous studies in younger children with PHIV[10,36] and adolescents with similar

Table 2 Comparison of Regional Brain Volumes, White Matter Damage, and Cerebral Blood Flow Between Adolescents With PHIV and Matched Controls

	PHIV	Controls					
	Mean ± SD or n (%)	Mean ± SD or n (%)	Beta	95% CI	*p* Value	[a]BH *p*	ES
Volumetry (L)							
GM	0.699 ± 0.06	0.689 ± 0.06	0.010	−0.02 to 0.04	0.486	0.681	0.16
WM	0.460 ± 0.05	0.467 ± 0.05	−0.008	−0.03 to 0.02	0.491	0.681	0.13
CSF	0.201 ± 0.05	0.215 ± 0.05	−0.007	−0.03 to 0.02	0.535	0.681	0.12
WM macrostructure							
WMH (any)	18 (58%)	16 (57%)			0.999	0.999	
WMH volume (mm^3) log	4.5 ± 1.5	3.9 ± 1.3	0.564	−0.43 to 1.56	0.256	0.554	0.38
WM microstructure							
FA	0.40 ± 0.02	0.41 ± 0.02	−0.012	−0.018 to −0.005	<0.001[b]	0.014[b]	0.46
MD (10^{-3} mm^2/s)	0.80 ± 0.03	0.79 ± 0.03	0.014	0.005 to 0.024	0.003[b]	0.014[b]	0.42
RD (10^{-3} mm^2/s)	0.62 ± 0.03	0.60 ± 0.03	0.02	0.007 to 0.03	0.002[b]	0.014[b]	0.43
AD (10^{-3} mm^2/s)	1.17 ± 0.02	1.16 ± 0.02	0.009	−0.0004 to 0.02	0.061	0.214	0.32
Cerebral blood flow (ratios)							
GM	0.93 ± 0.21	1.00 ± 0.22	−0.06	−0.17 to 0.05	0.277	0.554	0.32
WM	0.21 ± 0.06	0.23 ± 0.07	−0.02	−0.06 to 0.01	0.246	0.554	0.32
Caudate nucleus	0.92 ± 0.20	0.91 ± 0.24	0.007	−0.11 to 0.13	0.902	0.972	0.01
Putamen	0.82 ± 0.19	0.82 ± 0.22	0.007	−0.10 to 0.11	0.903	0.972	0.02
Thalamus	0.83 ± 0.22	0.88 ± 0.23	−0.05	−0.18 to 0.07	0.366	0.641	0.23

Abbreviations: AD = axial diffusivity; ES = effect size; FA = fractional anisotropy; GM = gray matter; MD = mean diffusivity; RD = radial diffusivity; WM = white matter; WMH = white matter hyperintensity.
Values in the second and third columns are either mean ± SD or number (percentage).
[a] BH *p* = adjusted *p* value with the Benjamini-Hochberg method. Any WMH presence was compared with the Fisher exact test. Differences were assessed by performing multivariable linear regression. We adjusted all models for age and sex. WMH (any) is defined as presence of any WMH lesion. WMH volume (mm^3) was logarithmically transformed to approach a normal distribution, and the regression model was additionally adjusted for WM volume. MD, RD, and AD were multiplied with 10^3 for interpretation purposes of coefficients. Cerebral blood flow is the ratio of individual CBF of ROI (in mL/100 g/min) to mean GM blood flow in controls (in mL/100 g/min). Effect size thresholds: |d| (0.01) = very small, |d| (0.2) = small, |d| (0.5) = medium, and |d| (0.8) = large.
[b] Significant *p* values.

age.[14] Our results add to the prior literature by demonstrating that these differences are unlikely to be due to adoption status. This is important as adopted children might endure psychosocial deprivation and malnutrition, both affecting WM integrity in children,[18,19] and previous studies did not match for or report differences in adoption or associated early-life characteristics.[10,14,36] WM maturation is at a critical stage in the first years of life,[17] thus coinciding with diagnosis and treatment initiation of perinatal HIV infection. Our findings might implicate that these differences in WM integrity could originate from that critical early-life period and reflect the inability of the brain to undo the lasting effects of early WM damage caused by HIV itself. This is substantiated by another study in children with PHIV that demonstrated that starting cART as early as possible may reduce HIV-associated WM damage.[37] Alternatively, lower WM integrity in our participants could hint at previous neurotoxic effects of cART.[38] However, we did not find an association between WM integrity and cART characteristics. Moreover, our previous longitudinal DTI assessment suggests that despite potential early damage, there is no progressive decrease of WM integrity in adolescents with PHIV while on effective treatment.[9] Together, our studies do not suggest that cART induced changes in WM integrity. FA is a very sensitive but not very specific biomarker of WM integrity; thus, the underlying pathophysiologic mechanisms remain unknown. On the other hand, higher MD and RD–in the presence of abnormal FA–have previously been associated with edema or necrosis and higher demyelination, respectively.[39] However, if these processes underlie our findings, we suspect them to be subtle, otherwise we would have detected larger WMH volume and/or more severe clinical symptoms, which is not the case in the adolescents with PHIV in our study.

Although PHIV had a lower IQ, we did not find an association between WM integrity and IQ, in contrast to a previous study in children without neurologic disease.[40] This could be a

Table 3 Association Analyses Between WM Microstructure and PHIV Characteristics

	FA		MD		RD	
	Beta (95% CI)	***p* Value**	**Beta (95% CI)**	***p* Value**	**Beta (95% CI)**	***p* Value**
HIV VL zenith log (copies/mL)	0.0004 (−0.005 to 0.006)	0.873	−0.001 (−0.01 to 0.006)	0.630	−0.002 (−0.01 to 0.006)	0.590
CD4$^+$ T-cell *Z* score nadir	0.004 (−0.007 to 0.01)	0.500	−0.01 (−0.02 to 0.01)	0.335	−0.01 (−0.03 to 0.01)	0.364
CDC HIV category						
B	−0.01 (−0.03 to 0.01)	0.274	0.01 (−0.02 to 0.04)	0.573	0.01 (−0.02 to 0.05)	0.457
C	−0.01 (−0.03 to 0.01)	0.346	0.01 (−0.02 to 0.04)	0.491	0.01 (−0.02 to 0.05)	0.450
Current cART use	0.01 (−0.02 to 0.05)	0.372	−0.01 (−0.05 to 0.04)	0.790	−0.01 (−0.06 to 0.04)	0.689
Undetectable VL at assessment	−0.003 (−0.03 to 0.02)	0.803	−0.005 (−0.04 to 0.03)	0.768	−0.002 (−0.04 to 0.03)	0.900
Age at treatment initiation (y)	0.001 (−0.001 to 0.003)	0.227	−0.001 (−0.003 to 0.001)	0.454	−0.001 (−0.004 to 0.002)	0.381
Duration of treatment (y)	−0.001 (−0.003 to 0.001)	0.230	0.001 (−0.001 to 0.003)	0.460	0.001 (−0.002 to 0.004)	0.385
IQ[a]	0.01 (−0.37 to 0.39)	0.272	0.39 (−2.4 to 3.2)	0.777	0.28 (−2.2 to 2.7)	0.814

cART = combination antiretroviral therapy; CDC = Centers for Disease Control and Prevention; FA = fractional anisotropy; GM = gray matter; MD = mean diffusivity; RD = radial diffusivity; VL = viral load; NA = no or minimal symptoms; B = moderate symptoms; C = severe symptoms or (brain) AIDS.
Association analyses using multivariable linear regression. We adjusted all models for age and sex. HIV viral load zenith was logarithmically transformed to approach a normal distribution. MD and RD were multiplied with 10^3 for interpretation purposes of coefficients.
[a] Despite this depiction, IQ was used as the outcome variable, and coefficients were multiplied with a factor 10^5 for interpretation purposes.

result of the fact that we used whole-brain averages of DTI parameters instead of investigating specific WM fiber bundles, or it might be that the reduced WM integrity in our participants was too small to affect cognitive function. Alternatively, the lack of association could be due to the narrow IQ range in our sample. In sum, the exact pathophysiologic mechanisms underlying the lower WM integrity and its clinical consequences remain to be completely elucidated.

We found no difference in the presence of any WMH between adolescents with PHIV and controls, which is in contrast with an earlier study from a partially overlapping cohort performed at a different time point.[10] Reasons for this difference are difficult to pinpoint but could include controlling for adoption status, sampling variation, or cohort differences, such as lower Centers for Disease Control and Prevention B and C classification in adolescents with PHIV in our study (15/35 vs 22/35). Our current finding of comparable WMH presence is in line with a previous study investigating children with PHIV in Zambia.[11] Yet, compared with that study, we found a higher mean WMH presence, which could be explained by the use of a higher MRI field strength in our study (3T vs 1.5T) resulting in a higher detection sensitivity of WMH.[41] In adults with HIV, there is contrasting evidence as to whether HIV is an independent contributor to WMH.[12,42] Surprisingly, we found a high percentage of controls with WMH (57%), which could reflect a better matched control group and indicate that WMH in adolescents with PHIV might not solely be a complication of a perinatal HIV infection. Moreover, WMH presence itself does not provide information about the extent of WMH. In this study, the relatively high presence of any WMH is accompanied with low absolute WMH volume in adolescents with PHIV (mean: 0.09 mL) and controls (mean: 0.05 mL), which probably impedes the clinical consequences at this moment. The lack of clinical consequences of WMH in children is also reported in studies investigating WMH presence in healthy cohorts[43] or pediatric patients with morbidities other than HIV (reported WMH presence up to 30%).[44,45] All studies had different MRI scan parameters, which may have affected WMH detection sensitivity. Altogether, follow-up is warranted to further understand the association between HIV and WMH, and its relevance in adolescents with PHIV could still emerge, as WMH have been associated with cognitive impairment at older age.[46] Finally, we explored the relation between cART and WMH. We found that older age at cART initiation or shorter duration of cART was associated with a higher WMH volume. Although HIV status was not associated with a significantly higher WMH volume in our cohort, these associations could, however, implicate that WMH in adolescents with PHIV partially originated from the period before treatment initiation. Therefore, starting cART as early as possible could mitigate the presence of a higher WMH volume in at least a portion of patients with PHIV.

We found comparable CBF between groups, in contrast to our previous cross-sectional study, which demonstrated higher CBF in WM and subcortical regions.[47] This difference could be inherent to the younger age in the previous cross-sectional assessment leading to interindividual physiologic effects in adolescents, because CBF swiftly declines in different brain regions during adolescence.[48] This is underscored by our longitudinal CBF assessment in which we found comparable changes in CBF between groups after a follow-up of 4.6 years.[16] Another explanation could be the variability in CBF, as CBF highly dynamic and could be influenced by many factors.[49]

This study has some limitations. Our small sample size reduced the generalizability of results and possibly hampered the detection of subtle associations. The cross-sectional design does not allow us to investigate causal effects or assess disease progression. Moreover, we assumed that adoption status may be associated with early-life adversity, such as malnutrition. However, we do not have medical records of children containing details before adoption; therefore, we do not know whether and to what extent the participants were affected. Although we did not assess HIV clades as a possible confounding factor to our results, another study showed WM microstructural damage to occur irrespective of the HIV clade.[50]

Despite effective treatment, adolescents with PHIV exhibit lower WM integrity compared with matched controls, regardless of international adoption status. These results underscore the neuropathogenicity of HIV or, to lesser extent, its treatment. Although GM, WM, and WMH volume and CBF in ROIs did not differ between groups, these findings warrant follow-up as adolescents with PHIV–frequently affected by cognitive impairment–grow into adulthood and experience lifelong disease and treatment.

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