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White matter microstructure among youth with perinatally acquired HIV is associated with disease severity

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Abstract

Objectives—We investigated whether HIV disease severity was associated with alterations in structural brain connectivity, and whether those alterations in turn were associated with cognitive deficits in youth with perinatally-acquired HIV (PHIV).

Design—PHIV youth (n=40) from the Pediatric HIV/AIDS Cohort Study (PHACS) (mean age: 16±2 yrs) were included to evaluate how current and past disease severity measures (recent/nadir CD4%; peak viral load) relate to white matter (WM) microstructure within PHIV youth. PHIV youth were compared to 314 controls from the Pediatric Imaging, Neurocognition, and Genetics (PING) study.

Methods—Diffusion tensor imaging and tractography were utilized to assess WM microstructure. Mediation analyses were conducted to examine whether microstructure alterations contributed to relationships between higher disease severity and specific cognitive domains in PHIV youth.

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Authors Dr.'s Williams, Malee, Yogev, Csernansky, Wang, Nichols and Sowell are leaders of the PHACS research team, and have been involved with funding, design, recruitment, and overseeing scanning/testing of the participants in the present manuscript. Dr. Uban designed, executed and drafted the analyses and manuscript. Dr. Herting provided training on methodology of DTI data. Dr.'s Herting and Gautam provided consulting for the analyses and interpretation of results, as well as editing drafts of the manuscript. Ms. Huo is a statistician working with Dr. Williams, and provided data organization and secure transfer, as well as consulting on analyses and reviewing drafts. Ms. Ajmera is a student of Dr. Sowell who helped with quality checking, pre-processing, preliminary data analyses, and editing of manuscript. Overall, this manuscript was a collaborative process, led by Dr. Uban who executed analyses and writing, and all listed authors played a significant role.

Conflict of Interest None of the authors have any conflicts of interest to report.

Results—Whole brain fractional anisotropy (FA) was reduced, but radial (RD) and mean (MD) diffusivity were increased, in PHIV compared to control youth. Within PHIV youth, more severe past HIV disease was associated with reduced FA of the right inferior fronto-occipital (IFO) and left uncinate tracts; elevated MD of the F minor; and increased streamlines comprising the left inferior longitudinal fasciculus (ILF). Associations of higher peak viral load with lower working memory performance were partly mediated by reductions in right IFO FA levels.

Conclusion—Our findings suggest that PHIV youth have higher risk of alterations in WM microstructure compared to typically developing youth, and certain alterations are related to past disease severity. Further, WM alterations potentially mediate associations between HIV disease and working memory.

Search terms

CD4 Cell Counts; Viral load; HIV; Diffusion tensor imaging (DTI); Diffusion tractography; cognition

Introduction

In the United States, many children with perinatally-acquired HIV (PHIV) have survived with combination antiretroviral treatment (cART). However, cognitive deficits have been observed among youth with PHIV compared to typically developing peers [1]. HIV affects central nervous system functioning, largely indirectly through damaging cytokines produced as a result of immune activation and inflammation [2, 3]. During replication, HIV destroys and leads to decreases in CD4+ immune T cells [4], a major indicator of disease severity. PHIV may produce unique effects on brain and cognition, considering HIV and associated immune changes likely impact early brain development.

Impaired cognitive performance [5-8] is associated with higher HIV RNA viral load (VL) and lower nadir CD4+ cell percentages [6, 7]. PHIV youth demonstrate deficits that are sensitive to HIV disease severity in working memory and processing speed [1]. In unaffected youth, white matter (WM) microstructure is important for plasticity underlying age-related increases in working memory capacity and processing speed [9, 10]. Thus, the present study investigated whether WM microstructure among youth with PHIV is altered compared to typically developing controls. We examined whether WM microstructure is affected by disease severity among PHIV youth, and if so, whether alterations in brain mediate the relationship between disease severity and poor cognitive performance.

WM is primarily made of organized myelinated axonal fiber bundles, which restrict water diffusion in a parallel direction to axons; increased maturation corresponds to increased restriction with age [11, 12]. Diffusion tensor imaging (DTI) measures this restriction of water diffusion [13]. Using tractography on DTI data, a three-dimensional estimate of WM tracts is generated [14], from which properties of water diffusion can be extracted. This technique's advantage includes not having to perform between-subject warping; making it a valuable tool for studies where disease may produce brain abnormalities [15]. One primary DTI variable is fractional anisotropy (FA) (i.e., magnitude of anisotropic diffusion). Increased FA levels likely reflect increases in myelination and/or axonal organization.

However, if myelinated nerve sheaths are affected in a patient population, it is possible that neurobiological compensation could lead to myelin hyperplasia, and higher FA could be related to poorer cognition [16]. Beyond FA, other DTI parameters can be estimated and examined along each tract, such as diffusivity: 1) along principle axis (axial (AD)); 2) averaged across two minor axes (radial (RD)); 3) averaged across three axes (mean (MD)); and estimated number of streamlines comprising a specific tract (i.e., number of fiber bundles). Using tractography-based along-tract statistics, it is feasible to detect localized effects associated with PHIV [15].

DTI parameters have been shown to reflect HIV disease severity in the adult rodent [17], macaque [18, 19] and human brain [20-31]. Lower FA was observed in HIV-infected compared to non-infected adults [22, 24, 25], and even higher FA in some WM regions [22]. In the infected adult, HIV progression has been associated with lower FA, but higher AD, RD and MD [20]. Additionally, T-cell alterations were associated with DTI measures in SIV-infected non-human primates [18]. The effects of HIV on WM microstructure may possibly differ in youth with PHIV, compared to those who acquired HIV in adulthood, given that HIV exposure and cART occur when significant brain development is occurring. Sarma and colleagues [32] examined volume of WM in PHIV youth and identified WM atrophy that differed from those observed with adult-acquired HIV. To our knowledge, no study to date has utilized tractography to examine associations between disease severity markers and microstructural properties at specific points along WM tracts among PHIV youth.

The present study aims to address gaps in the literature by investigating WM microstructure: 1) in PHIV compared to typically developing youth; 2) as a function of disease severity markers; and 3) as a potential mediating factor between disease severity and cognitive performance within PHIV youth. Given overall lower FA values in HIV-infected adults [22, 24, 25], we hypothesized that PHIV youth would exhibit decreased FA compared to non-affected youth, and that greater disease severity among PHIV youth would relate to even lower FA values, which in turn, would contribute to lower cognitive performance.

Methods

Study population

PHIV—40 PHIV adolescents were selected from the Adolescent Master Protocol (AMP) study of the NIH Pediatric HIV/AIDS Cohort Study (PHACS) at a single site (Northwestern University, Chicago, IL). AMP is a prospective study designed to define the impact of perinatal HIV exposure, PHIV and cART on youth. Institutional review boards at Northwestern and Harvard approved the study. Parents, legal guardians or youth aged 18 years or older provided written informed consent for research participation; youth over 12 years provided assent.

Controls—A control group was generated using frequency-matching from the Pediatric Imaging, Neurocognition, and Genetics (PING) study (http://pingstudy.ucsd.edu/welcome.html) magnetic resonance imaging (MRI) database from five sites (MGH; Sackler

Institute; UH; Yale; UCLA). Adolescents were frequency-matched by age, sex, and scanner type, totaling 314 PING youth.

Disease Markers and Cognitive Functioning in PHIV youth

Disease and cognitive functioning were examined among PHIV youth only. Information regarding clinical diagnoses and laboratory results, including CD4% and plasma HIV RNA concentration (viral load; VL), was obtained by medical chart abstraction at AMP study visits (see [8] for more detail). The lowest known CD4 percent (nadir CD4%) and highest known VL ("peak VL") were collected. We considered three measures of disease severity: nadir CD4%, recent CD4%, and peak VL. Recent HIV RNA levels were not utilized in analyses due to lack of variability (only 6/40 (15%) had recent VLs above 400 copies/mL (range: <40-92,000)). Age at the time of acquisition of past disease markers was utilized as a covariate in analyses. Age at time of scan was highly correlated with age at assessment of current disease markers and cognitive testing, and was utilized as a second covariate in analyses. Biological sex was utilized as a covariate in all analyses. The mean interval between scanning and: (1) assessment of current disease markers was 1.8 months (SD±3.5 months); and (2) cognitive testing was 0.9 months (SD±6.9 months).

The Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV) (6-16 years), and the Wechsler Adult Intelligence Scale, Fourth Edition (WAIS-IV) for 17+ [33, 34] were used to evaluate cognitive performance in PHIV youth. We targeted: 1) Working memory (WMI); and 2) Processing speed (PSI) indexes.

Image Acquisition

Acquisition protocols were standardized across the PHACS and PING imaging sites (as described in [35]). At the PHACS Chicago location, whole brain diffusion weighted imaging data were acquired on a single 3-Tesla Siemens Tim Trio MRI scanner with 12-channel head coil. Diffusion weighted volumes (gradient encoding pulses applied in 30 directions, b0=1000 s/mm^2, in-plane matrix of 96×96, axial slices with 2.5mm thickness and 90° angle, TE=86000ms, TR=172000ms) and one non-diffusion weighted volume (b=0s/mm^2) were acquired. Similar protocols were used for PING 3T Siemens imaging sites with the exception of an 8-channel head coil.

Image Processing and Analyses

Between Group Analyses—Quality control and processing of PHIV and control youth were executed with the PING processing portal (http://pingstudy.ucsd.edu/investigators.html). Briefly, average whole brain FA was calculated using FMRIB Software Library (FSL) DTI toolbox, and an atlas-based method for labeling and characterizing tracts based at UCSD (described in detail in [35]. To investigate group differences in whole brain FA, AD, RD, and MD, ANOVA was performed in R software [36], adjusting for sex and age at scan. *Post-hoc* effect size estimates (Cohen's *d*) were calculated using each group's mean and standard deviation [37].

Within-PHIV Analyses—In order to investigate WM microstructure *along* major WM tracts in relation to disease severity and cognition, standard DTI preprocessing [38] and

Trackvis [39] tractography were utilized followed by in-house scripts previously described [15]. Eddy current and motion distortions were corrected, a six parameter tensor model of diffusion was fit to the data to estimate voxelwise FA, AD, RD, and MD using a12-parameter affine to register to standardized space (MNI152 template). Next, whole-brain brute-force, atlas-based tractography was performed using the Diffusion Toolkit v0.6 (http://www.trackvis.org/dtk). This software implements Fiber Assignment by Continuous Tracking (FACT) algorithm [14] to generate deterministic streamlines by iteratively moving from voxel to voxel along the direction of maximal diffusion. The following constraints of (1) a whole-brain mask, (2) an FA threshold of 0.15 and (3) a tract-dependent turning angle threshold of 60° were used to reduce biologically implausible fibers. Successful tracts were defined as those with ≥1 streamline. Nine atlas-based WM tracts were identified using a multi-ROI approach established by [40] (Table 2). FA, AD, RD and MD at multiple points were parameterized along 9 major WM tracts using the along-tract mapping toolbox [15], MATLAB [41] and R software [36], as well as number of streamlines comprising each tract. *Post-hoc* effect size estimates using r^2 were provided when appropriate to identify how well each model explained the proportion of total variation of outcomes.

To examine within-subject relationships with FA/AD/RD/MD and disease severity or cognition, linear-mixed effect modeling was conducted in R [36], with DTI parameters along each tract as repeated measures. Analyses testing associations between streamline numbers and disease severity or cognition utilized linear modeling. To confirm previous findings of associations between disease severity and cognition [7] in the current sample, linear modeling was utilized using sex, age at peak VL, and age at cognitive testing as covariates. Significant associations were followed up with simple Pearson's correlation. Peak VL was not normally distributed (negatively skewed), and therefore was log transformed (base 10) prior to statistical analyses. Potential bivariate outliers were removed for secondary analyses to confirm statistical findings.

Mixed effect modeling was utilized to assess the association of biological disease markers with (i) DTI parameters (FA/AD/RD/MD) along each WM tract and (ii) streamline number of each tract, with sex, age at peak VL and age at scan as covariates. All linear mixed effect-modeling results (uncorrected $p<0.05$) were followed by permutations (n=1000) to obtain adjusted p-values, utilizing a threshold-free cluster enhancement algorithm [42]. Statistical trends were reported (corrected $p<0.10$).

To investigate the potential mediating role of WM microstructure alterations in associations observed between disease markers and cognition in PHIV youth, mediation analyses were conducted (mediation package in R [43]). Following analyses with brain and disease severity, mean values of FA/AD/RD/MD and streamline number were calculated from significant points along the tract and used in mediation analyses with disease severity and cognitive indices (WMI/PSI).

In order to reduce the likelihood of motion as a confounder in our analyses, motion during the scans was quantified in PHIV youth with FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLMotionOutliers) using frame-wise displacement [44]. Linear mixed-effects modeling was utilized to assess associations between motion and: (1) age at scan; (2) sex; and (3)

markers of disease severity. As expected, there was a significant association between motion and age at scan with younger participants displaying increased motion, hence age at scan was used as a covariate in all analyses reported. There were no other significant associations with motion in PHIV youth.

Results

Study Population

40 PHIV youth and 314 Control youth from PING ranged in age from 11.6 to 20.7 years (Table 1). Within the PHIV youth, nadir CD4% and peak VL were not significantly correlated. For between group comparisons, two DTI scans could not be processed through the PING portal from the PHIV cohort, resulting in a sample of n=38 for PHIV versus Control between-group analyses. For within-group analyses with disease severity measures and cognition, all 40 PHIV youth were included.

Differences in whole brain DTI parameters in PHIV compared to control youth

Relative to typically developing control youth, PHIV youth exhibited significantly lower FA (by 8.3%; d=1.78), higher RD (by 10.9%; d=1.44), and higher MD (by 5.1%; d=1.13) averaged across the whole brain (all $p<0.001$), after adjustment for age at scan and sex. There were no statistical differences between groups in AD (d=.00).

Associations between disease markers and cognition among PHIV youth

In linear models adjusted for age at scan and sex, as well as age at peak VL, positive associations were found between peak VL and WMI ($p<0.10$; r^2=.10) and PSI ($p<0.05$; r^2=.17), with higher log peak VL associated with poorer performance on both cognitive domains. There were no significant associations between nadir CD4% (all r^2=.03-.04) or recent CD4% (all r^2=.009-.06) and cognitive indices.

Association of disease markers with DTI parameters among PHIV youth

A summary of findings can be found in Table 2

Fractional anisotropy (FA): Significant negative associations were found between peak VL and FA along the right IFO. Specifically, peak VL was negatively associated with FA (r=-0.45, $p<0.01$; r^2=.20), and this effect was observed only in the mid-to-anterior portion of the right IFO (Figure 1A). Additional significant associations were found between peak VL and FA along the left UNC ($p<0.05$), where peak VL was negatively correlated with FA (r=-0.56, $p<0.001$; r^2=.31) only in the lateral-to-mid portions of the left UNC (Figure 1B). There were no significant associations between nadir CD4% or recent CD4% and FA.

Axial diffusivity (AD): Significant associations were found between nadir CD4% and AD along the forceps minor (Fminor) ($p<0.10$), where higher nadir CD4% was associated with lower AD (r=-0.39, $p<0.05$; r^2=.15) only in the midline, crossing through the genu (Figure 2A). There were no significant associations between peak VL or recent CD4% and AD.

Radial diffusivity (RD): There were no significant associations between peak VL, nadir CD4% (r^2=.00) or recent CD4% (r^2=.00) and RD.

Mean diffusivity (MD): Significant associations were found between peak VL and MD along the Fminor ($p<0.05$), where a higher peak VL was associated with higher MD values ($r=0.54$, $p<0.001$; $r^2=.29$) only in the midline, crossing through the genu (Figure 2B). After removal of a potential bivariate outlier (e.g., high MD and high Peak viral load), the association remained significant ($r=0.32$, $p<0.05$; $r^2=.10$). There were no significant associations between nadir CD4% ($r^2=.00$) or recent CD4% ($r^2=.00$) and MD.

Streamlines: Significant positive associations were observed between peak VL and streamline number in the left ILF ($r=0.30$, $p=0.05$; $r^2=.09$) (Figure 2C). There was a statistical trend for the association between nadir CD4% and streamline number in the left ILF ($F(4,35)=2.48$, $p=0.06$), where lower nadir CD4% was associated with more streamlines, but the follow-up correlation was not significant ($r=-0.16$; $r^2=.02$).

Mediation analyses with disease severity, brain and cognition: Mediation analyses were conducted when cognition was significantly correlated with disease severity, disease severity was significantly correlated with DTI measures, and cognition was correlated with DTI measures at $p<0.10$. These conditions were met for the following: (1) peak VL, right IFO FA with WMI and PSI ($p<0.10$); (2) peak VL, left UNC FA with PSI ($p<0.10$); and (3) peak VL, Fminor streamline number with PSI ($p<0.10$). Out of these four 3-way associations, only one significant mediated effect was observed. Specifically, higher peak VL (mediated by reduced IFO FA) was associated with reduced WMI performance (total effect=61%), and the indirect effect of reduced FA accounted for 59% of the total effect of high peak VL.

Discussion

Significant differences in whole brain WM microstructure were observed in PHIV compared to typically developing youth (represented by overall lower FA and higher RD and MD). Within PHIV youth, advanced disease severity (e.g., lower nadir CD4% and higher peak VL) was associated with certain WM microstructure alterations in the expected direction, based on comparisons with typically developing youth in the present study. Specifically, higher peak VL was associated with lower FA (left UNC, right IFO), higher MD (F minor) and higher streamline counts (left ILF), whereas lower nadir CD4% was associated with higher AD (F minor). Additionally, association with working memory performance was partially mediated by reductions in FA along the right IFO. Higher disease activity may impact organization and/or myelination of underlying WM microstructure, suggesting a possible decrease in WM integrity with life-long HIV and cART exposure.

Alterations in whole brain DTI parameters observed in PHIV compared to control youth parallel those observed in HIV+ adults

PHIV youth exhibited whole brain reductions in FA, but higher RD and MD values compared to controls. The findings with FA and MD parallel those with adults, where reductions in FA [22, 24, 25] but increases in MD were observed in HIV+ compared to control adults [21, 26]. This suggests that axonal organization and/or myelination may be impacted by both perinatal and adulthood HIV. Further, markers of disease progression in

HIV+ adults were associated with lower FA, but higher AD, RD and MD [20]. RD may be a proxy for myelination, with higher RD reflecting less myelination of tracts [20]. Higher MD values observed among children with developmental delays [45], may be a sensitive indicator of developmental problems. It is possible that higher MD, observed in PHIV youth and HIV+ adults [20], may represent neurodegeneration of WM: where a loss in neurons during neurodegeneration, corresponds to increased water molecules, possibly resulting in higher MD [46]. Supporting this hypothesis, smaller WM volumes have been observed among PHIV youth [32]. Adults with Hepatitis C virus (HCV) infection also exhibit lower FA and higher MD compared to healthy controls [47], suggesting that different types of chronic peripheral infections and inflammation may result in similar profiles of WM microstructure abnormalities.

Both peak VL and CD4 counts were associated with streamline number of the left ILF, where indices of increased disease severity were related to more streamlines (proxy for myelinated fiber bundles). The ILF connects occipital and temporal lobes; therefore, higher streamline number may be a compensatory mechanism to counteract the negative impact of HIV and/or cART exposure on the ILF. However, working memory deficits are observed in this and prior [1, 7] studies; thus, an increase in ILF width may not completely rescue cognitive deficits. Further, associations between both disease severity markers and FA, AD, RD and MD were observed in tracts that connect to the frontal lobes, suggesting that HIV may impact frontal tracts to a greater extent than posterior tracts. Lower nadir CD4 percentages were associated with higher AD of the F minor, again suggesting that the genu and frontal cortex may be particularly susceptible to PHIV. Previous studies have found similar alterations in WM microstructure in the corpus collosum of adult non-human primates and humans, where HIV+ participants exhibited low FA [24, 48] and high MD [18, 21, 25, 26, 49]. As WM in the frontal lobes are the last to fully mature [50], these regions could be more susceptible to HIV-related factors compared to posterior tracts. Interestingly, adult HIV is also associated with greater frontal compared to posterior brain alterations [20-24, 27].

VL reflects viral activity and replication, whereas CD4% reflects immune suppression; but, we observed associations of both measures with WM alterations. Higher peak VL related to lower FA along the right IFO and left UNC and higher MD along the F minor. The IFO and UNC connect the frontal and temporal lobes. Thus, peak VL, but not nadir CD4%, relates to FA alterations in tracts that are implicated in integration of auditory and visual cortices with the prefrontal cortex (IFO) and connections between temporal and frontal regions (UNC). The IFO connects the frontal, temporal and occipital lobes [51]. The UNC connects part of the limbic system (hippocampus, amygdala) with frontal regions, such as the orbitofrontal cortex. Reductions in FA along the right IFO and left UNC may reflect less axonal organization and/or myelination of these slowly maturing tracts. It is possible that perinatally acquired HIV produces a long-lasting increase in MD, and in the corpus callosum (midline F minor) as observed among PHIV youth.

Similar to Smith et al., [7], we found that peak VL was associated with reduced working memory and processing speed performance among PHIV youth. Lower FA in the right IFO and left UNC were associated with poorer processing speed, but lower FA in these tracts did

not statistically mediate the relationship between higher disease severity and lower cognitive performance. However, lower FA in the right IFO mediated the relationship between higher disease severity and poorer working memory. Previous studies have demonstrated that working memory may be susceptible to HIV infection during brain development [1]. Associations of peak VL with FA along this tract may be evidence of cART effectiveness, along with corpus collosum alterations. This needs to be confirmed in a larger cohort of PHIV youth, as the present study had low power to detect brain-cognition associations given the relatively small sample size.

Limitations of the present study include that the control and patient samples were scanned at different sites. However, scanner type, acquisition protocol and pre-/post-processing methodologies were identical, and controls were frequency-matched by age and sex to PHIV youth, allowing for meaningful preliminary between group comparisons. It is important to note that this limitation only applies to between group comparisons with PHIV and control youth, and not the disease associations among PHIV youth, which are compelling on their own. Second, the cross-sectional nature of the present study limits our ability to assess the impact of HIV on brain development over time (e.g., change). Third, we assessed only a limited range of cognitive domains, and future studies should use a more comprehensive cognitive battery.

Conclusion

Overall, greater HIV-related disease severity early in life may impact organization and/or myelination of underlying WM microstructure, suggesting a possible decrease in WM integrity with life-long HIV. Alterations in brain development may contribute to cognitive deficits observed among PHIV youth. Understanding the impact of HIV disease severity on WM integrity further emphasizes the importance of medication adherence in order to control viral load early in life, as well as provide potentially useful clinical tools for evaluating cART efficacy during a dynamic period of brain development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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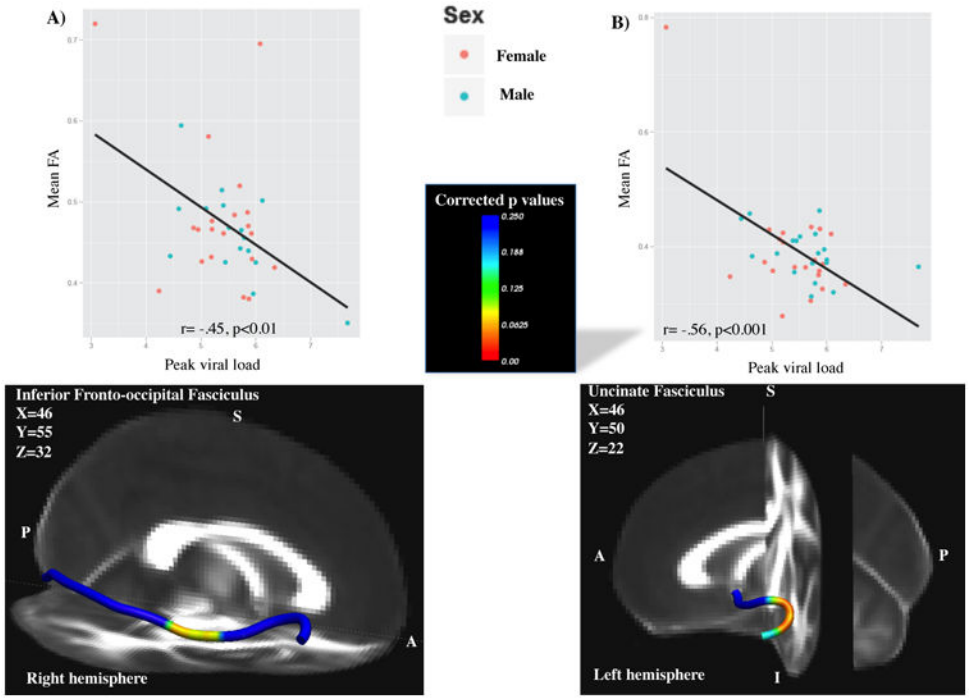

Figure 1.

Higher peak VL was associated with lower FA levels in the A) right inferior fronto-occipital fasciculus (IFO); B) left uncinate fasciculus (UNC). Statistical representation of corrected p values overlaid onto 1mm FA standard image. A=anterior, I=inferior, P=posterior, R=right.

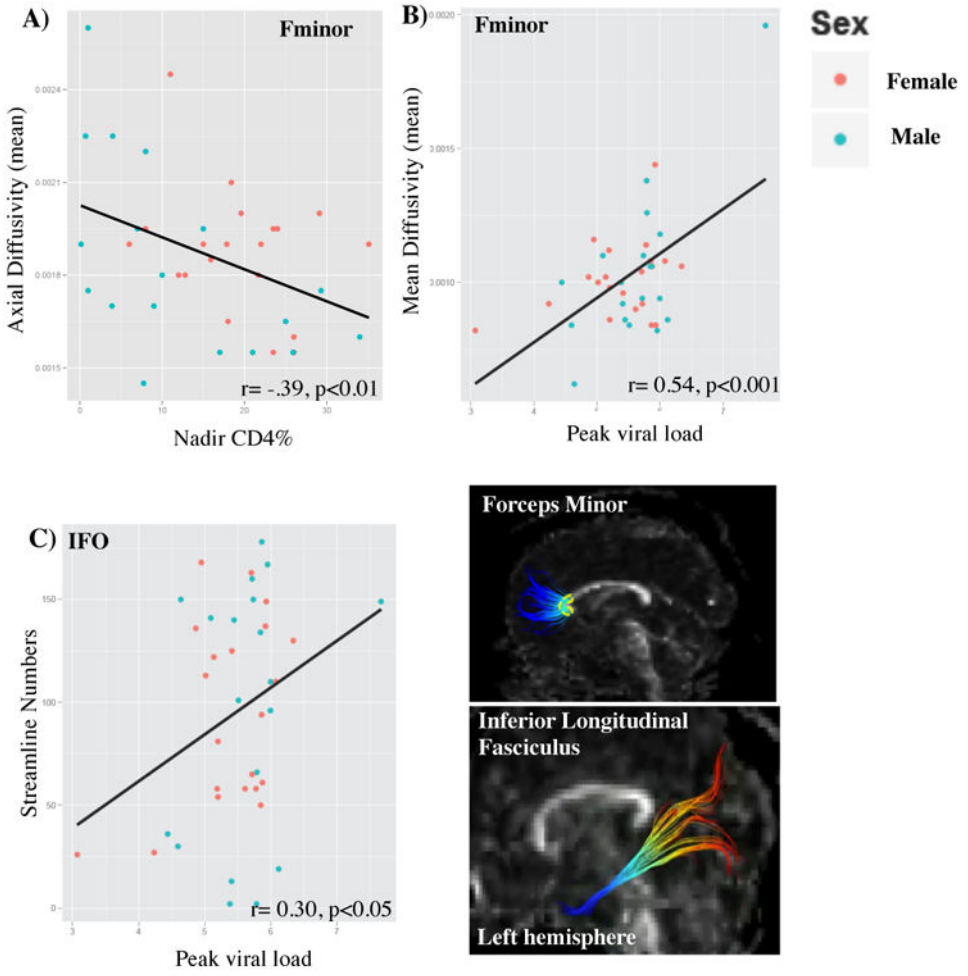

Figure 2.

Associations between disease severity and AD, MD and streamline number. A) Greater disease severity was associated with higher AD values in the genu of the F minor (outlined in yellow). B) Greater disease severity was associated with higher MD values in the genu of the F minor. C) Greater disease severity was associated with overall more streamlines in the left ILF. AD=axial diffusivity; MD=mean diffusivity; ILF= inferior longitudinal fasciculus.

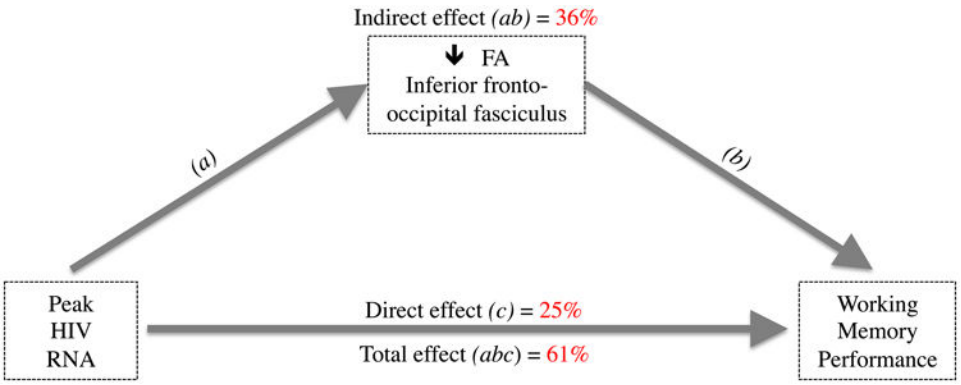

Figure 3.

Mediation model for Peak HIV RNA, Right IFO FA and working memory. The association between higher *Peak HIV RNA levels* and poorer working memory performance was mediated, in part (36%), by lower FA in the right IFO. Indirect effect represents the mediating factor (FA), and direct effect represents the association between *Peak HIV RNA* and working memory, excluding FA.

Table 1

Sample characteristics describing youth with PHIV and Control youth.

		PHIV (PHACS)	Control (PING)
General	*Sample size*	40	314
	Age at time of scan	16.7 ± 2.4 (11.6 - 20.7 years)	16.1 ± 2.7 years (11.6 - 20.7 years)
	Male:Female (%female)	19:21 (52%)	155:159 (50%)
Disease severity variables	*Nadir CD4%*	16.0 ± 9.5	
	Age at nadir CD4%	6.8 ± 5.4 years	
	Recent CD4%	33.5 ± 11.5	
	Log Peak viral load (copies/mL)	5.4 ± 0.7	
	Age at peak viral load	4.0 ± 4.6 years	
	Recent viral load >400 copies/mL	6 (15%)	
	CDC 'C' classification	9 (22.5%)	
Cognition	*Working Memory Index*	87.5 ± 16.8	
	Processing Speed Index	95.3 ± 14.1	
	Cognitive Proficiency Index	90.2 ± 16.0	

Mean ± Standard Deviation or N (%), as appropriate. CDC= Centre for Disease Control; Cognitive (IQ) scores are normalized (mean=100±15).
Note: Disease severity variables and Cognition were not assessed in Control participants.

Table 2

Summary of *significant* associations between disease severity and DTI measures among youth with PHIV.

White Matter Tract	Disease severity measure	DTI parameter	Hemisphere	r value	p value
Forceps minor (Fminor)	Nadir CD4%	AD	n/a	-0.39	p<0.05
Forceps minor (Fminor)	Nadir CD4%	MD	n/a	0.54	p<0.001
Inferior fronto-occipital fasciculus (IFO)	Peak viral load	FA	right	-0.45	p<0.01
Inferior longitudinal fasciculus (ILF)	Peak viral load	Streamline count	left	0.30	p=0.05
Uncinate fasciculus (UNC)	Peak viral load	FA	left	-0.56	p<0.001

Associations between disease severity markers and FA, AD, MD and number of streamlines among youth with PHIV (adjusted p values following 1,000 permutations are reported). Pearson r represents directionality of association, for both the left and right hemispheres. There were no significant associations between Recent CD4% and DTI parameters. FA: fractional anisotropy; DTI: diffusion tensor imaging. AD: axial diffusivity; MD: mean diffusivity.