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Research Paper

Cortical structural changes related to early antiretroviral therapy (ART) interruption in perinatally HIV-infected children at 5 years of age

Emmanuel C. Nwosu [a,f,*], Martha J. Holmes [a,f], Mark F. Cotton [b], Els Dobbels [b], Francesca Little [c], Barbara Laughton [b], Andre van der Kouwe [d,e], Ernesta M. Meintjes [a,f], Frances Robertson [a,f]

[a] UCT Medical Imaging Research Unit, Division of Biomedical Engineering, Department of Human Biology, Faculty of Health Sciences, University of Cape Town, South Africa
[b] Family Centre for Research with Ubuntu, Department of Paediatrics & Child Health, Tygerberg Children's Hospital and Faculty of Health Sciences, Stellenbosch University, Cape Town, South Africa
[c] Department of Statistical Sciences, Faculty of Sciences, University of Cape Town, South Africa
[d] A.A. Martinos Centre for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Charlestown, MA, USA
[e] Department of Radiology, Harvard Medical School, Boston, MA, USA
[f] Neuroscience Institute, Faculty of Health Sciences, University of Cape Town, South Africa

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ABSTRACT

ART interruption in children can occur especially in resource-limited settings for reasons including poor adherence, stock-outs, ART intolerance of non-pediatric formulas and pill size, as well as ultimately to test for HIV remission. Although early ART initiation is now standard of care in pediatric HIV management, very little is known on the effect of early ART initiation or subsequent interruption on brain development. This study aimed to investigate the effect of ART interruption on brain cortical thickness (CT) and folding in a subset of children from the Children with HIV Early antiRetroviral therapy (CHER) trial cohort who all started ART before 18 months of age. CHER participants in the neuroimaging follow-up study had magnetic resonance (MRI) scans on a 3T Siemens Allegra brain scanner at age 5.44 ± 0.37 years. MR images were processed using the automated cross-sectional stream in FreeSurfer v6.0 and vertex wise comparisons of CT and local gyrification indices (LGIs) were performed between HIV+ children and HIV- controls, as well as between HIV+ children on interrupted or continuous ART and controls. HIV+ children (n = 46) showed thicker cortex than HIV- children (n = 29) in bilateral frontal and left temporo-insular regions but lower LGIs in left superior and bilateral medial orbitofrontal cortex extending into rostral anterior cingulate. Children on interrupted ART (n = 21) had thicker cortex than HIV- controls in left frontal and right insular regions, but children on continuous treatment (n = 25) showed no difference from controls. Children on both interrupted and continuous ART showed region-specific alterations in LGI relative to controls. Cortical folding appears more sensitive than CT to early life events including early ART and interruption. However, immune health resilience in children can translate to long term preservation of morphometric brain development, especially for those on early and continuous treatment.

Introduction

Untreated perinatal HIV infection (PHIV) has severe consequences that include immune depletion, severe intercurrent and opportunistic infections and both neurodevelopmental and cognitive deficits (Weber et al., 2017; Sánchez-Ramón et al., 2002, 2003). High viral load in children with PHIV is associated with neurological consequences including HIV encephalopathy (HIVE) (Sánchez-Ramón et al., 2002, 2003), and infants with PHIV are at greater risk for cytomegalovirus (CMV) co-infection (Adachi et al., 2018; Kfutwah et al., 2017; Slyker et al., 2009), itself a major cause of hearing loss and cognitive impairment (Adachi et al., 2018; Barbi et al., 2006; Manicklal et al., 2013).

International pediatric antiretroviral therapy (ART) guidelines (World Health Organization (WHO), 2008; ART Guideline, 2008; Paediatric European Network for Treatment of AIDS (PENTA) Steering Committee: PENTA, 2009) have recommended early ART initiation as standard of care since 2008. Early ART aims to achieve viral load suppression within the shortest possible time to prevent mortality and

* Correspondence to: Human Biology Department, Faculty of Health Sciences, Anzio Road, Observatory, Cape Town, 7935, South Africa.
E-mail address: emmanuel.c.nwosu@gmail.com (E.C. Nwosu).

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severe clinical events in infancy (Ndongo et al., 2018; Crowell et al., 2015; Phongsamart et al., 2014; Violari, 2008). Starting ART within the first year of life minimizes neurological insult (Weber et al., 2017; Crowell et al., 2015; Puthanakit et al., 2013; 2012). Early ART has increased survival amongst PHIV infants and provides protection from at least the short-term clinical and neuropsychological consequences of HIV (Laughton et al., 2018; Cotton et al., 2013; Brahmbhatt et al., 2014). However, these benefits may be affected by ART interruption.

Several factors predispose to ART interruption. These include poor adherence, stock-outs, ART intolerance due to lack of pediatric ART formulation and pill size (Dubrocq and Rakhmanina, 2018; Ananworanich et al., 2016; Wamalwa et al., 2016; Ebonyi et al., 2015; Biadgilign et al., 2009). While HIV remission research has employed an intensive monitored antiretroviral pause as the ultimate test for remission (Ananworanich et al., 2016; Li et al., 2015), long-term ART interruption may have severe adverse effects (Wamalwa et al., 2016; Ananworanich et al., 2006; Danel et al., 2006; Montserrat et al. 2017; Ananworanich et al., 2016).

ART interruption affects children and adults differently (Lewis et al., 2017; Calin et al., 2016; Ananworanich et al., 2016). In adults and adolescents, since ART plays dual roles of both treatment and prevention of further HIV replication (Calin et al., 2016; Tubiana et al., 2002; Ananworanich et al., 2016), long-term interruption may lead to rapid viral rebound that may not be reversed within at least the first two years of ART resumption, with possible neurological consequences (Calin et al., 2016; Montserrat et al., 2017) and immune health remaining lower than on continuous treatment (Ananworanich et al., 2006; Danel et al., 2006; Lewis et al., 2017). On the other hand, since early treatment prevents many adverse effects associated with pediatric HIV (Lewis et al., 2017; Wamalwa et al., 2016; Ananworanich et al., 2016; Bunupuradah et al., 2013a), interruption may have little effect on children in the short term (Lewis et al., 2017). Infants' immune systems are also more plastic and dynamic than those of older children and adults (Lewis et al., 2017; Wamalwa et al., 2016) and children restarting ART after a planned interruption are likely to have CD4 recovery similar to children on continuous treatment (Bunupuradah et al., 2013a; Lewis et al., 2017). However, ART interruption may be especially disadvantageous if it occurs during critical developmental cycles.

In the Children with HIV Early antiRetroviral (CHER) trial, early ART prevented a decline in CD4 count, but did not lead to complete recovery to the CD4 counts seen in uninfected children (Lewis et al. 2017). ART interruption was associated with CD4 T-cell decline, which on resumption of treatment returned to the original pre-interruption levels. This study concluded that immune health before ART initiation is an important indicator of overall CD4 level and immune health during subsequent development. Early ART initiation helps to stabilize immune health, to the extent that any disturbance due to ART interruption will recover once treatment is resumed. A CHER sub-study showed neurodevelopmental outcomes similar to uninfected controls at 5 years in the participants on ART interruption arms (Laughton et al., 2018). While timing of first ART initiation and duration on ART before interruption are considered relevant determining factors for neurodevelopmental delay (Ananworanich et al., 2016; Puthanakit et al., 2013; Bunupuradah et al., 2013b), the effect of ART interruption on brain morphometry development in children has not been studied.

As part of an ongoing longitudinal study, we have been following the children from the CHER trial who are resident in Cape Town from age 5 years with neuroimaging and neurodevelopmental assessment. The present study presents a cross-sectional investigation of the effect of early ART, followed by interruption, on brain morphometry in these children at age 5 years – the earliest age at which neuroimaging data were acquired. We aimed to find out how ART interruption influences brain morphometry – specifically cortical thickness (CT) and cortical folding, measured via local gyrification indices (LGIs) – in early treated PHIV children.

We hypothesized that early ART would prevent structural damage with little or no morphometric difference between HIV infected (HIV+) and HIV uninfected (HIV-) children. We also predicted no difference between PHIV children with ART interruption and children on continuous treatment, due to the resilience of children's immune systems, early ART initiation and relatively short duration of ART interruption in most children.

Methods

Study participants

Study participants were 83 South African children (53 HIV+, 30 uninfected controls (HIV-); mean age ± SD: 5.44 ± 0.37 years; 38 boys) from a follow-up study of the CHER trial conducted at the Family Centre for Research with Ubuntu at Tygerberg Children's Hospital, Cape Town. In the CHER trial, HIV+ infants with CD4% ≥ 25% were randomized at age 6–12 weeks to receive immediate limited duration ART followed by planned interruption (either after 40 or 96 weeks of treatment), restarting when clinical and/or immunological criteria were met, or to start continuous ART only when they developed clinical symptoms or CD4 depletion below 20% (25% in the first year) as per treatment guidelines at the time (World Health Organization (WHO), 2006). All HIV+ children started ART before 76 weeks of age and received comprehensive immunological and clinical follow-up. The ART regimen received by participants consisted of Zidovudine (ZDV) + Lamivudine (3TC) + Lopinavir-ritonavir (LPV/r, Kaletra®) (CHER, 2010; Violari et al. 2008; Cotton et al. 2013) which was the South African government-regulated standard of care at that time. The HIV- control group was recruited from an interlinked vaccine trial (Madhi et al., 2010) and comprised both children born to HIV infected mothers (HIV exposed uninfected, HEU; n = 17) and to HIV- mothers (HIV unexposed, HU; n = 13). The extended revised version of the Griffiths Mental Development (GMDS) scales was performed at 5 years of age to assess neurodevelopment (Laughton et al., 2018). The GMDS for children of 2–8 years is made up of locomotor, personal-social, hearing and language, eye and hand co-ordination, task performance (speed and precision), and practical reasoning sub-scales (Jacklin & Cockcroft, 2012; Luiz et al., 2006). We present summary scores for performance (EQ) and practical reasoning (FQ) subscales as well as an aggregate of the subscales (GQ).

Image acquisition and analysis

The children completed T1-weighted high-resolution structural magnetic resonance imaging (MRI) at age 5 years on a Siemens (Erlangen, Germany) 3 Tesla Magnetom Allegra dedicated brain scanner located at the Cape Universities Brain Imaging Centre (CUBIC). Children were scanned in sagittal orientation using a single channel head coil and a 3D echo planar imaging (EPI) navigated (Tisdall et al., 2012) multi-echo magnetization prepared rapid gradient echo (MEMPRAGE; van der Kouwe et al, 2008) sequence (FOV 224 × 224 mm^2, TR 2530 ms, TI 1160 ms, TEs = 1.53/3.19/4.86/6.53 ms, bandwidth 657 Hz/px, 144 slices, 1.3 × 1.0 × 1.0 mm^3) that prospectively corrects for motion during the scan. To limit motion due to restlessness, children watched a movie via a mirror and rear projection screen during scanning. Scans were performed without sedation according to protocols approved by the Faculty of Health Sciences Human Research Ethics Committees of both the Universities of Cape Town and Stellenbosch. Parents/guardians provided written informed consent and the children gave oral assent. Viral loads were suppressed (<400 copies/mL) at time of scanning in 93.5% (43/46) of the HIV+ children who provided usable imaging data.

MR images were manually checked and those with poor visual quality were excluded. The remaining images were processed using the automated stream in FreeSurfer version 6.0 (https://surfer.nmr.mgh.harvard.edu/fswiki/ReleaseNotes). FreeSurfer analyses were done on a Linux Ubuntu version 16.04 LTS machine. DICOM image files were

Table 1
Demographic data for all participants (N = 75).

	HIV+	HIV-	t[b] or $\chi 2$[c]	p-value
Sample size (*N*)	46	29	–	–
Age at scan (years)	5.3 ± 0.2	5.6 ± 0.4	-4.00	< 0.001
Number of males (%)	20 (44%)	16 (55%)	0.56	0.45
Birth weight (g)	3066 ± 416	3005 ± 586	0.56	0.58
Estimated total intracranial volume (ETIV) (cm^3)	1373 ± 98	1361 ± 111	0.50	0.62
Participants' MRI scan quality measure (Euler number)				
Right hemisphere (mean, median, IQR)	−165, −154, 60	−157, −150, 56	−0.61	0.54
Left hemisphere (mean, median, IQR)	−160, −146, 60	−150, −135, 70	−0.80	0.43
GMDS[a] assessed at age 5 years				
Performance (EQ) scores	73.9 ± 9.8	76.5 ± 18.5	−0.83	0.41
Practical reasoning (FQ) scores	76.8 ± 8.3	76.2 ± 10.2	0.27	0.78
Sub-scales aggregate (GQ) scores	83.3 ± 6.1	83.1 ± 8.1	0.11	0.91

All values are mean ± standard deviation except where indicated otherwise.
[a] Griffiths Mental Development Scales – Extended Revised: 2–8 years.
[b] Independent two-tail *t*-test.
[c] Chi-squared test.

converted and reconstructed with FreeSurfer's cross-sectional "recon-all" pipeline, which registers each subject's data to a template, performs intensity normalization, gray and white matter segmentation, and tessellation of the gray/CSF and white/gray matter boundaries (Dale et al., 1999). Cortical thickness is then measured as the shortest distance between the pial and the white matter surfaces (Dale et al., 1999) and the local gyrification index (LGI) is calculated as the ratio of the amount of cortex buried within the sulcal folds to the amount of cortex on the outer visible surface (Schaer et al., 2012). FreeSurfer outputs were manually checked for processing and segmentation errors. Outputs were manually edited and reconstructed for minimal errors in cortical segmentation but were excluded completely for major errors. FreeSurfer's Euler number, which summarizes the topological complexity of the reconstructed cortical surface, was used to assess the structural image quality, as recommended by Rosen et al. (2018). Reconstructed surface data were sampled to the FreeSurfer average subject template for vertex-wise analysis. The smoothing kernel for cortical thickness analyses was 10 mm full-width, half-maximum (FWHM) of the spatial gaussian filter; no smoothing was used for LGIs.

Vertex-wise analysis of cortical thickness and LGIs

Cross-sectional group comparisons of CT and LGIs over the whole brain were performed between HIV+ children and HIV- controls using FreeSurfer's mri_glmfit function. Children who had interrupted ART and those on continuous ART were compared and separately compared to the HIV- controls.

All analyses were performed adjusting for age at scan. Moreover, since sex-related differences in cortical and sub-cortical structures are reported in brain development (Wierenga et al., 2017; Fisher et al. 2016), we adjusted for sex. All vertex-wise results were thresholded at an initial uncorrected threshold of $p < 0.05$, after which cluster-wise correction for multiple comparisons was performed using pre-computed Monte Carlo simulation for a two-tailed test. We report clusters surviving a cluster-wise corrected threshold of $p < 0.05$, with no adjustment for the two hemispheres. All further statistical analyses were done in R version 3.4.1 (https://www.r-project.org/) under RStudio version 1.0.143 (2016).

Table 2
Developmental and clinical data for HIV+ children (N = 46).

	ART-Interrupted	Continuous ART	t[c] or W[d]	p-value
Sample size (N)	21	25	–	–
GMDS[a] assessed at age 5 years				
Performance (EQ) scores	72.71 ± 10.47	74.87 ± 9.30	−0.79	0.43
Practical reasoning (FQ) scores	75.62 ± 9.49	77.68 ± 7.21	−0.90	0.37
Sub-scales aggregate (GQ) scores	82.50 ± 6.73	83.92 ± 5.48	−0.84	0.41
Participants' MRI scan quality measure (Euler number)				
Right hemisphere (mean, median, IQR)	−175, −170, 56	−158, −144, 61	−1.12	0.27
Left hemisphere (mean, median, IQR)	−150, −142, 56	−169, −155, 65	1.35	0.18
Clinical data at study enrolment (age 6–8 weeks)				
CD4 count ($cells/mm^3$)	1934 ± 1080	1777 ± 824	0.60	0.55
CD4% ($cells/mm^3$)	35 ± 9	33 ± 10	0.56	0.58
CD4/CD8 ratio	1.4 ± 0.7	1.3 ± 0.8	0.73	0.47
CD8 count ($cells/mm^3$)	1464 ± 645	1706 ± 988	−1.01	0.32
Viral load at enrolment				
High (>750,000 copies/mL), n (%)	9 (42.86%)	16 (64%)	–	–
Low (400–750,000 copies/mL), n (%)	12 (57.14%)	9 (36%)	–	–
Suppressed (<400 copies/mL), n (%)	0 (0%)	0 (0%)	–	–
Clinical data at scan (age 5 years)				
CD4 count ($cells/mm^3$)	1072 ± 360	1317 ± 675	−1.60	0.12
CD4% ($cells/mm^3$)	35 ± 6	37 ± 8	−0.61	0.55
CD4/CD8 ratio[a]	1.2 (0.4) [0.5 – 30.2]	1.2 (0.8) [0.5 – 4.2]	313	0.54
CD8 count ($cell/mm^3$)	920 ± 398	1069 ± 553	−1.10	0.28
Viral load at scan (Age 5 years)				
High (>750,000 copies/mL)	0 (0%)	0 (0%)	–	–
Low (400–750,000 copies/mL)	1 (5%)	2 (8%)	–	–
Suppressed (<400 copies/mL)	20 (95%)	23 (92%)	–	–
Other				
Age at ART initiation (weeks)[a,b]	8.3 (2.6) [6.6–12.0]	20.9 (25.9) [6.3–75.7]	191	0.01
ART interruption timing (40 weeks / 96 weeks)	15 / 6	–	–	–
Age of first viral load suppression (weeks)[a]	34.1 (16.8) [30.6–213.3]	47.6 (50.4) [29.1–169.7]	262	0.13
Duration of ART interruption (weeks)[a]	44.1 (53.9) [5.7–299.4]	0	4.51	< 0.001
Cumulative duration on ART (weeks)	211 ± 54	252 ± 24	−3.75	< 0.001
Nadir CD4% ($cells/mm^3$)	20 ± 6	20 ± 6	−0.11	0.91
US Centres for Disease Control and Prevention (CDC) classification, n (%)				
A	4 (19%)	0 (0%)	–	–
B	4 (19%)	4 (16%)	–	–
Severe B	0 (0%)	8 (32%)	–	–
C	13 (62%)	11(44%)	–	–
Unknown	0 (0%)	2 (8%)	–	–
HIV encephalopathy diagnosis	4 (19%)	3 (12%)	–	–

Values are mean ± standard deviation, except where indicated otherwise.
[a] Median (IQR) [range].
[b] All children in the ART-Interrupted group initiated ART before age 12 weeks; in the continuous ART group, 12 children initiated ART before age 12 weeks and 13 children after age 12 weeks.
[c] Independent two-tail *t*-test.
[d] Mann–Whitney *U* test.

Table 3
Regions showing cortical thickness and gyrification differences between groups.

Comparison	Cortical thickness Region	Size (mm^2)	MNI peak Coordinates	Effect size at cluster peak[a]	Gyrification Region	Size (mm^2)	MNI peak Coordinates	Effect size at cluster peak[a]
Infection								
HIV+ vs HIV-	↑ L superior frontal gyrus	3748	(-9, 42, 25)	0.54	↓ L superior frontal gyrus	1512	(−7, 19, 58)	−0.19
	↑ R caudal middle frontal			0.49	↓ L medial orbitofrontal		(−16, 25, −22)	−0.12
	↑ L superior temporal / insula	880	(46, 24, 32)	0.59	extending into rostral anterior cingulate.	1138		
		1445	(-56, 3, -11)		↓ R medial orbitofrontal extending into rostral anterior cingulate	1664	(15, 35, 21)	−0.12
Treatment interruption								
ART interrupted vs Continuous ART	↓ L lateral occipital	812	(-40, -84, -14)	-0.25	↓ L precuneus extending into	2971	(−13, −68, 49)	−0.21
					superior parietal	866	(25, −79, 37)	−0.19
					↓ R superior parietal			
ART interrupted vs HIV-	↑ L rostral middle / superior frontal	974	(-30, 30, 28)	0.28	↓ L precuneus extending into	1789	(−9, −74, 45)	−0.13
		763	(35, -20, 1)	0.36	superior parietal	722	(16, 44, 9)	−0.15
	↑ R insula				↓ R rostral and caudal anterior cingulate	1334	(25, −87, -9)	0.17
					↑ R lateral occipital			
Continuous ART vs HIV-	No differences				↓ R superior frontal gyrus, posteriorly	518	(8, 3, 58)	−0.31
						588	(15, −39, 72)	−0.24
					↓ R superior parietal lobule			
					↑ L fusiform	936		0.24
					↑ R rostral middle frontal/pars triangularis / pars orbitalis	832	(−41, −53, −12) (50, 34, −7)	0.36
					↑ R lateral occipital			
						469	(36, −77, −12)	0.21

↑ greater; ↓ lower; L left; R right; [a]Unstandardized regression coefficient for group.

Results

Sample characteristics

We excluded data for 7 HIV+ children due to inaccurate automated cortical segmentation; one HIV- child was excluded due to incomplete MRI acquisition. Demographic data for all participants included in this analysis are presented in Table 1, and clinical data for HIV+ children in Table 2. On average, children in the HIV+ group were about 3.6 months younger at the time of scanning than the HIV- controls. Among the HIV+ children, 21 children (46%) had ART interrupted and restarted. Age at ART initiation and cumulative duration of ART were lower in the ART-interrupted group. Table 2 also shows US Centres for Disease Control and prevention (CDC) classification of HIV severity for study participants, where HIV symptom severity increases from category A (mild) to C (severe) (CDC, 1993).

Table 3 summarizes regions showing cortical thickness and gyrification differences between groups.

Effects of HIV status on cortical thickness and gyrification

HIV+ children had significantly thicker cortex than HIV- controls in bilateral frontal and left superior temporal/insular regions (Fig. 1a and b), and lower gyrification in an overlapping left superior frontal region, as well as bilateral medial orbitofrontal regions extending into rostral anterior cingulate cortex (Fig. 1c and d).

Effects of ART interruption on cortical thickness and gyrification

Within the HIV+ group, children whose treatment was interrupted had thinner cortex compared to those with continuous treatment in a left lateral occipital region (Fig. 2a and b), and lower gyrification in bilateral parietal regions (Fig. 2c and d). Post hoc regression analyses of peak CT and LGI in these clusters revealed that effects of ART interruption remain significant (L lateral occipital cortex CT: $\beta = 2.59$, $p < 0.001$; L superior parietal/precuneus LGI: $\beta = 0.12$, $p = 0.02$; R superior parietal LGI:: $\beta = 0.10$, $p = 0.01$) after adjustment for potential confounding by age at ART initiation and cumulative duration of ART.

Compared to HIV- controls, HIV+ children whose treatment had been interrupted had thicker cortex in left superior/rostral middle frontal and right insular regions (Fig. 3a and b), while HIV+ children on continuous treatment showed no significant difference in cortical thickness from HIV- controls.

The ART-interrupted group showed lower gyrification than controls in left precuneus and right rostral and caudal anterior cingulate regions, but higher gyrification in a right lateral occipital region (Fig. 3c and d). Children on continuous treatment showed lower gyrification posteriorly in the right superior frontal gyrus and superior parietal lobule than HIV- controls, but higher gyrification in left fusiform, and right rostral middle frontal/pars triangularis/pars orbitalis and lateral occipital regions (Fig. 4a and b).

Discussion

This study is the first to investigate the effect of early ART interruption on neuromorphometric development in young virally suppressed HIV+ children on treatment. HIV+ children had thicker cortex than HIV- controls in frontal regions of both hemispheres and a left superior temporo-insular region. Since children in whom treatment had been interrupted had thicker cortex in similar left frontal and right temporo-insular regions while those on continuous ART showed no CT differences to HIV- controls, thicker cortex in these regions may be attributable, in part, to treatment interruption. When compared to the continuous treatment group directly, children in whom treatment had been interrupted demonstrated thinner cortex only in a left lateral occipital region.

HIV+ children had lower gyrification than HIV- controls bilaterally in rostral anterior cingulate and medial orbitofrontal regions, as well as a left superior frontal region similar to that where they had thicker cortex. Of these, the lower gyrification in the right anterior cingulate

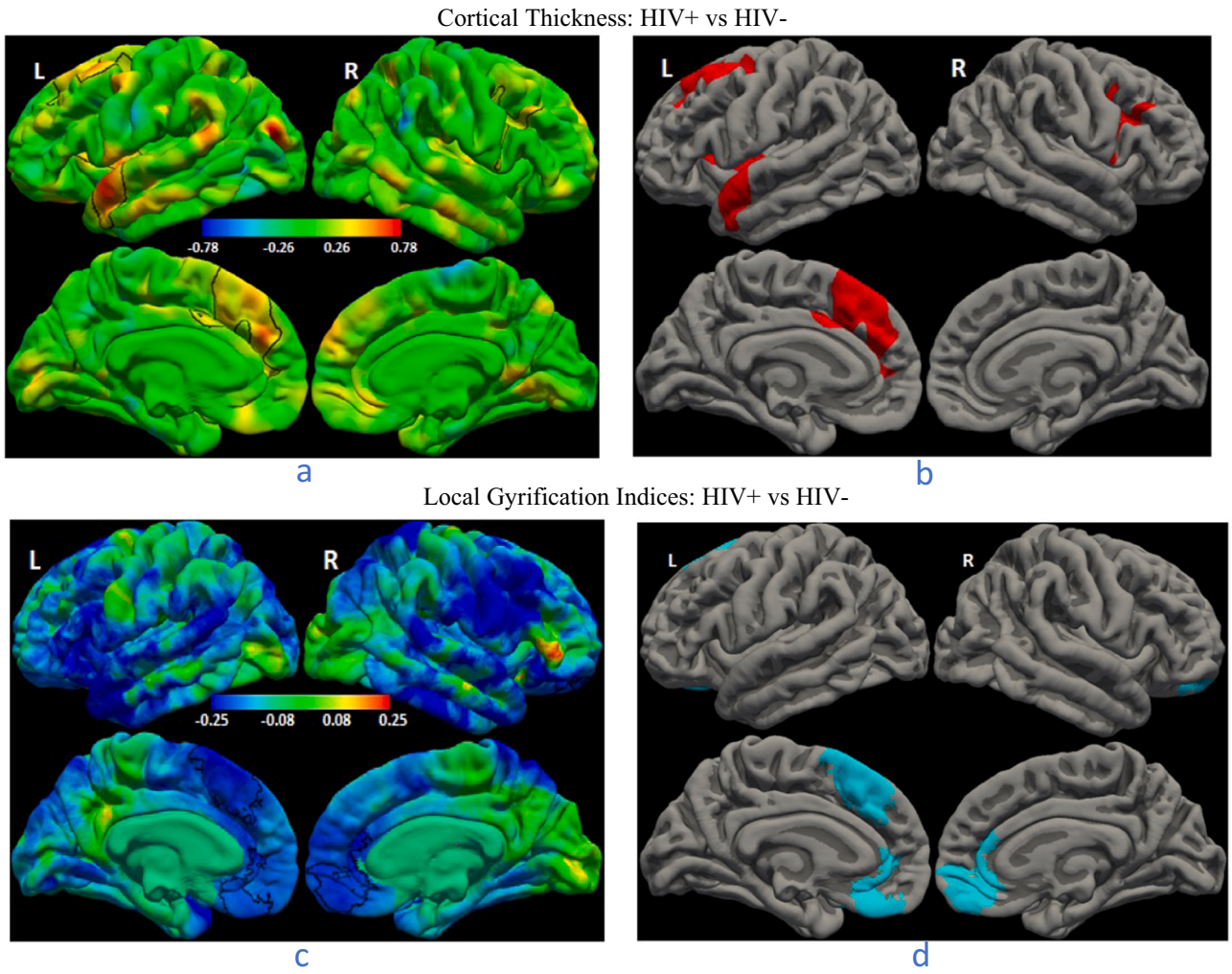

Fig. 1. Results from vertex-wise regression of (*Top row*) cortical thickness and (*Bottom row*) local gyrification indices against HIV status, controlling for sex and age at scan. Images on the left are colour maps of unstandardized parameter estimates for HIV status, and images on the right show only clusters where the groups differed significantly (cluster size corrected threshold of $p < 0.05$). Positive regression coefficients (red/yellow) indicate HIV+> controls (HIV-) and negative coefficients (cyan/blue) indicate HIV+< controls. The colour bar scales indicate the effect size (Cohen's *d*) and apply to both lateral (top) and medial (bottom) views. Top row: Frontal (left superior frontal/cingulate/precentral/caudal middle frontal and right caudal middle frontal) and left superior temporal / insula regions where HIV+ children have thicker cortex compared to HIV- controls. Bottom row: The left superior frontal and bilateral medial orbitofrontal / rostral anterior cingulate regions where HIV+ children have smaller local gyrification indices compared to HIV- controls.

was also evident in the subset whose treatment had been interrupted, but not in those on continuous ART. Children with ART interruption additionally demonstrated lower gyrification than controls as well as children on continuous ART, in a left precuneus-superior parietal region – a region not seen in the HIV+ group as a whole nor continuously treated children – and higher gyrification in a right lateral occipital region. In continuously treated children, we found higher gyrification in a similar right lateral occipital region, as well as left fusiform and right middle frontal regions, and lower gyrification posteriorly in the right superior frontal gyrus and right superior parietal lobule. Notably, the regions showing lower and higher gyrification in the continuously treated children were also evident in the ART-interrupted group, although mostly not significant.

Effects of HIV

Similar to our findings here of lower medial frontal LGIs and thicker right lateral frontal, left medial frontal, and left temporo-insular cortex at age 5 years in HIV+ children relative to HIV- controls, we previously reported lower bilateral medial parietal and right temporal gyrification in children from the same cohort at age 7 (Nwosu et al., 2018). At age 7 years, thicker cortex was only observed in a left inferior lateral occipital region.

Although to date few studies have examined gyrification in HIV, Hoare et al. (2018) recently also reported lower gyrification in the right lateral occipital gyrus and left inferior temporal gyrus in HIV+ adolescents than uninfected controls. Lewis-de los Angeles, C.P. (2017) similarly observed lower gyrification in perinatally HIV-infected youths in bilateral lateral and medial frontal regions and medial temporal lobe. Lower regional cortical folding due to perinatal HIV infection is therefore a consistent finding across studies, both in early childhood and into adolescence. The reason for the variation in regions showing these differences in different age groups is unclear and may be related to spatial variation in the rate of development of gyrification during childhood and varying disease course during and preceding this period.

The period of greatest increase in gyrification is thought to be after 24 weeks gestation (White et al., 2010), continuing postnatally till its peak at age 2 years (Li et al., 2014; Raznahan et al., 2011). Findings on gyrification during childhood and adolescence have been varied. An early study found increasing cortical complexity from 6 to 16 years in bilateral inferior and left superior frontal cortices (Blanton et al., 2001), while others have reported decreasing mean global gyrification after 3–4

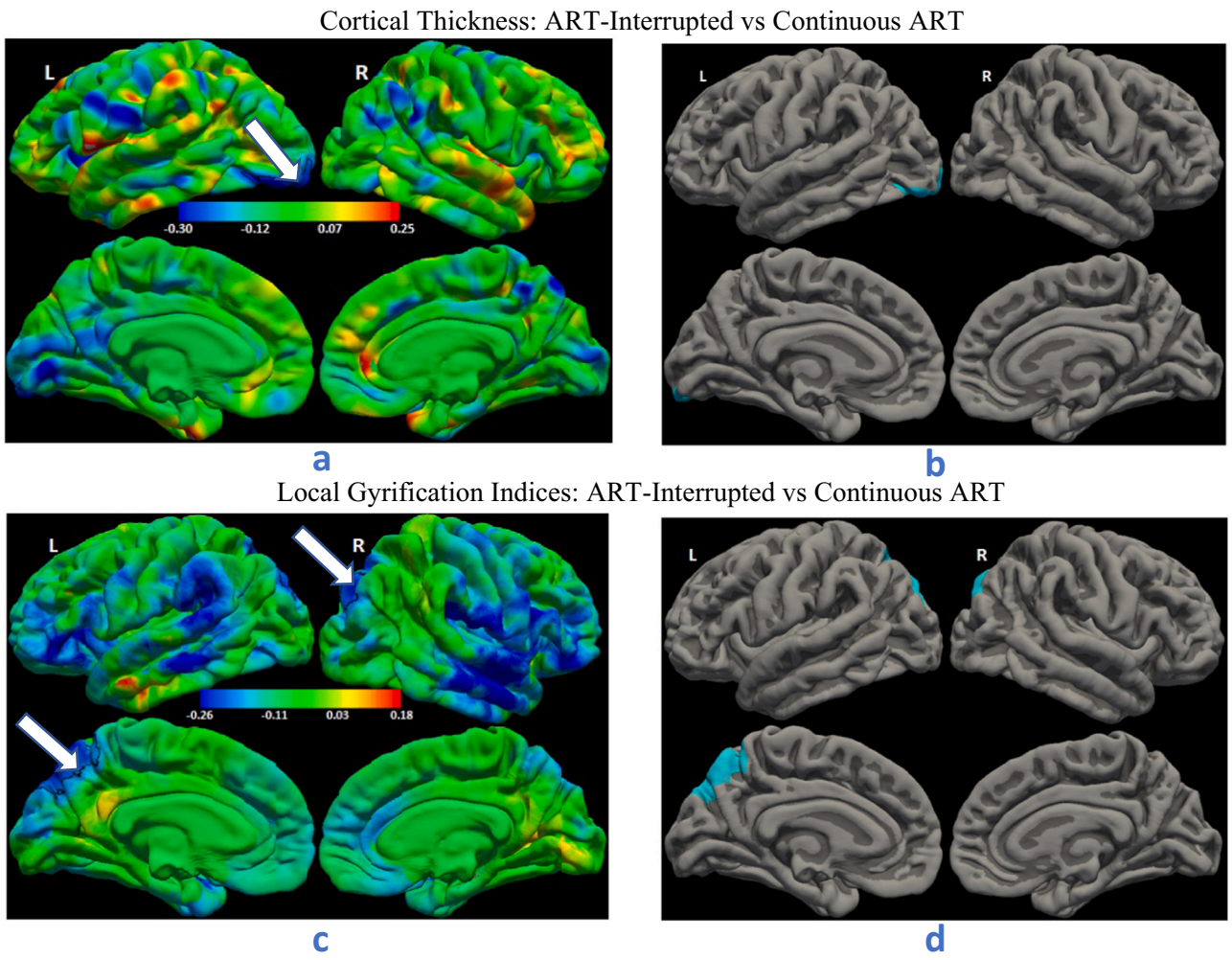

Fig. 2. Results from vertex-wise regression of (*Top row*) cortical thickness and (*Bottom row*) local gyrification indices against group (ART-Interrupted vs Continuous ART), controlling for sex and age at scan. Images on the left are colour maps of unstandardized parameter estimates for group, and images on the right show only clusters where the groups differed significantly (cluster size corrected threshold of $p < 0.05$). Positive regression coefficients (red/yellow) indicate ART-Interrupted > Continuous ART, and negative coefficients (cyan/blue) indicate ART-Interrupted < Continuous ART. The colour bar scales indicate the effect size (Cohen's *d*) and apply to both lateral (top) and medial (bottom) views. Top row: The left lateral occipital region where children with ART interruption have thinner cortex compared to children receiving continuous ART. Bottom row: The bilateral parietal regions where children with ART interruption have lower gyrification compared to those receiving continuous ART.

years of age (Raznahan et al., 2011; Cao et al., 2017), or regional decreases from pre-adolescence through young adulthood, including in later maturing frontal and temporal regions (Su et al., 2013; Klein et al., 2014; Aleman-Gomez et al., 2013). Recently, a study in children aged 1–6 years found age-related gyrification increases in some regions over this period and decreases in others (Remer et al., 2017), suggesting that gyrification is still increasing in some regions during early childhood, while it has already peaked and is decreasing in others.

The regionally lower gyrification in HIV+ children than controls seen here could therefore result from a failure or delay of gyral formation in early life, or an acceleration of the hypothesized normal reduction in LGI observed during childhood maturation from about 4 years, and into adulthood (Raznahan et al., 2011; Cao et al., 2017). Accelerated aging is known to occur in HIV+ adults and adolescents (Horvath et al., 2018) and a similar phenomenon in HIV+ children might increase the rate at which gyrification changes during maturation.

If gyrification failure or delay is the main cause of observed differences, one would expect deficits to persist or resolve at an older age, while differences resulting from accelerated aging would presumably appear or get progressively worse with age. Neither process alone can, however, explain our findings of lower gyrification at age 5 years in distinctly different regions than reported previously in the same children at age 7 years (Nwosu et al., 2018) – widespread lower medial frontal gyrification at 5 compared to more localized medial parietal and temporal regions at 7. Instead, our findings suggest that both processes play a role, albeit in different regions. In rostral anterior cingulate, for example, where LGI increases significantly from ages 1–6 years (Remer et al., 2017), LGI reductions appear to be due to HIV-related developmental delay that resolves by age 7 years. In contrast, in middle and superior temporal cortices where LGI decreases from 1 to 6 years in typically developing children (Remer et al., 2017), HIV-related lower LGI seen at age 7 years may be attributable to accelerated aging. Notably, we did see non-significant lower gyrification in temporal regions at age 5 years, consistent with this interpretation. In left superior frontal and bilaterally in medial orbitofrontal and paracentral cortices, LGI appears to plateau over the period from 1 to 6 years, changing by ±3% or less (Remer et al., 2017), making it more difficult to unambiguously attribute differences observed in these regions to either process, although the fact that lower LGI seen at 5 are not evident at 7 years, and vice versa, suggest developmental delay in the former and accelerated aging in the latter.

It is interesting that the medial frontal regions where HIV+ children

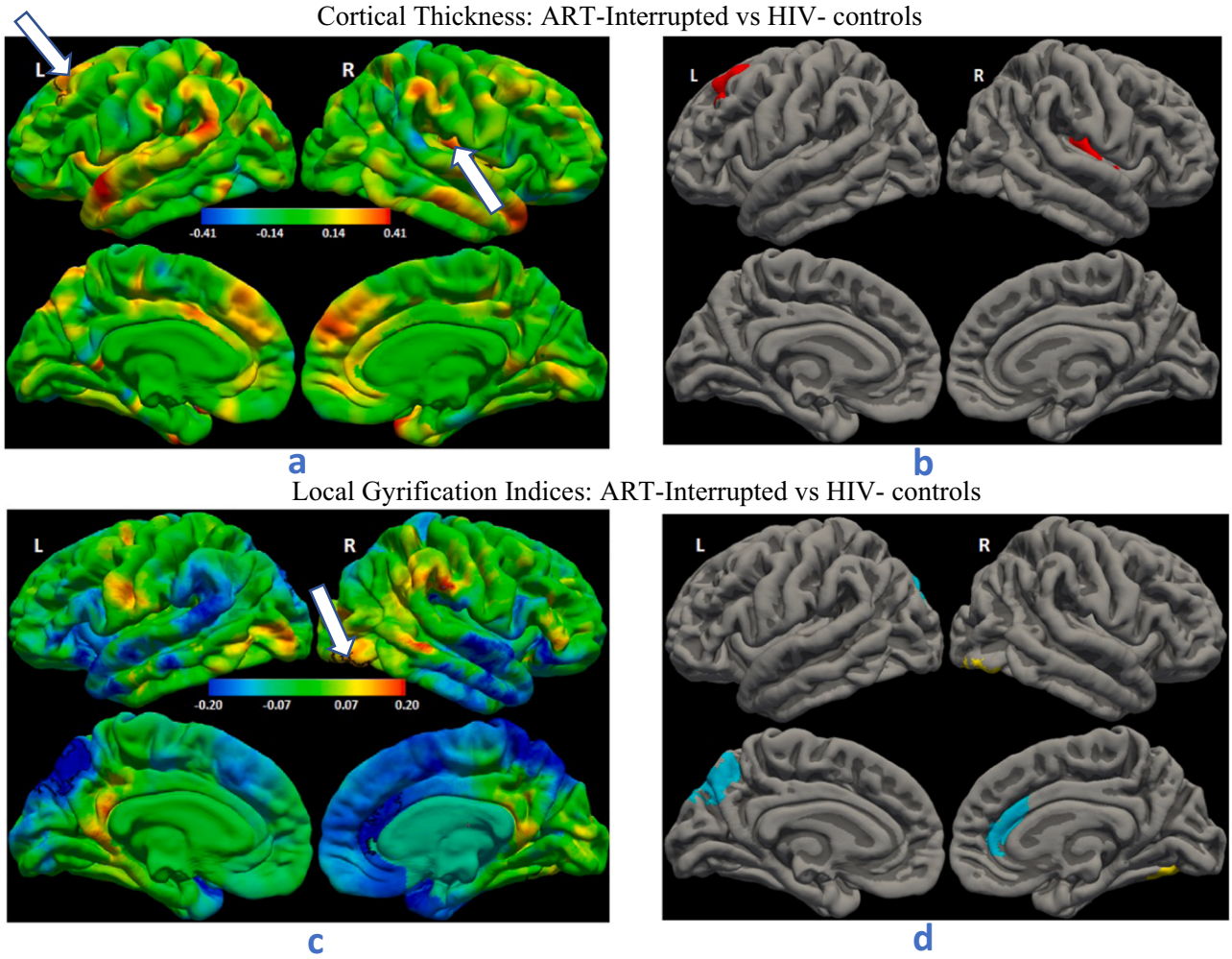

Fig. 3. Results from vertex-wise regression of (*Top row*) cortical thickness and (*Bottom row*) local gyrification indices against group (ART-Interrupted vs HIV-controls), controlling for sex and age at scan. Images on the left are colour maps of unstandardized parameter estimates for group, and images on the right show only clusters where the groups differed significantly (cluster size corrected threshold of $p < 0.05$). Positive regression coefficients (red/yellow) indicate ART-Interrupted > controls (HIV-), and negative coefficients (cyan/blue) indicate ART-Interrupted < controls (HIV-). The colour bar scale indicates the effect size (Cohen's *d*) and applies to both lateral (top) and medial (bottom) views. Top row: The left superior / rostral middle frontal region and right insula where children with ART interruption have thicker cortex compared to HIV- control children. Bottom row: The left precuneus and right rostral and caudal anterior cingulate regions where children with ART interruption have lower gyrification compared to HIV- control children, and the right lateral occipital region (indicated with a white arrow) showing higher gyrification.

showed lower gyrification than controls at age 5 correspond closely with the regions where at age 7 we found lower gyrification in children who initiated ART before 12 weeks compared to later treatment (Nwosu et al., 2018). This supports that LGI differences in this region are due to alterations in early life gyral formation.

HIV-related thicker cortex at age 5 years involved more regions, covered greater surface area and were observed in both hemispheres, compared to age 7 years where only a small left inferior lateral occipital region demonstrated thicker cortex (Nwosu et al., 2018). Similar to gyrification, neither developmental delay nor HIV-related accelerated aging can satisfactorily account for the disparate and anatomically distinct regions where cortical thickness differences were seen at these two ages, suggesting that both processes play a role.

Thicker cortex at age 5 in left superior frontal, left superior temporo-insular and right caudal middle frontal cortices, regions that show linear thinning from 1 to 6 years of age in typical development (Remer et al., 2017), point to HIV-related developmental delay. Since no differences were found in these regions at 7 years, sustained viral load suppression and maintained immune health appear to normalise CT development.

In contrast to our findings, recent studies have shown region-specific thinner as well as thicker cortex due to perinatal HIV infection. In a pediatric cohort on ART, Yadav et al. (2017) reported thinner cortex in the bilateral postcentral and right superior temporal regions, and thicker cortex in the left rostral middle frontal and right rostral anterior cingulate regions. Lewis-de los Angeles, C.P. (2017) similarly reported thinner cortex in the bilateral frontal and temporal lobes and left cingulate gyrus, but thicker cortex in the occipital lobe, in a cohort of HIV+ adolescents all but one of whom were on ART. Yu and Gao (2019) reported thicker left occipital cortex (middle and inferior gyri) and right olfactory sulcus, but thinning in middle temporal gyrus, temporal pole and orbitofrontal regions in HIV+ adolescents on ART compared to HIV-exposed uninfected adolescents.

It is interesting that we found only one region, namely left superior frontal cortex, that demonstrated both thicker cortex and lower gyrification in HIV+ children. For the remainder, HIV-related cortical thickness differences were observed on lateral surfaces and gyrification differences medially. This finding is similar to that of Klein et al. (2014) who found age-related cortical thinning and LGI decreases from 12 to 24 years in largely distinct regions, and no associations of LGIs with CT in the 8 regions showing the largest age-dependent LGI decreases.

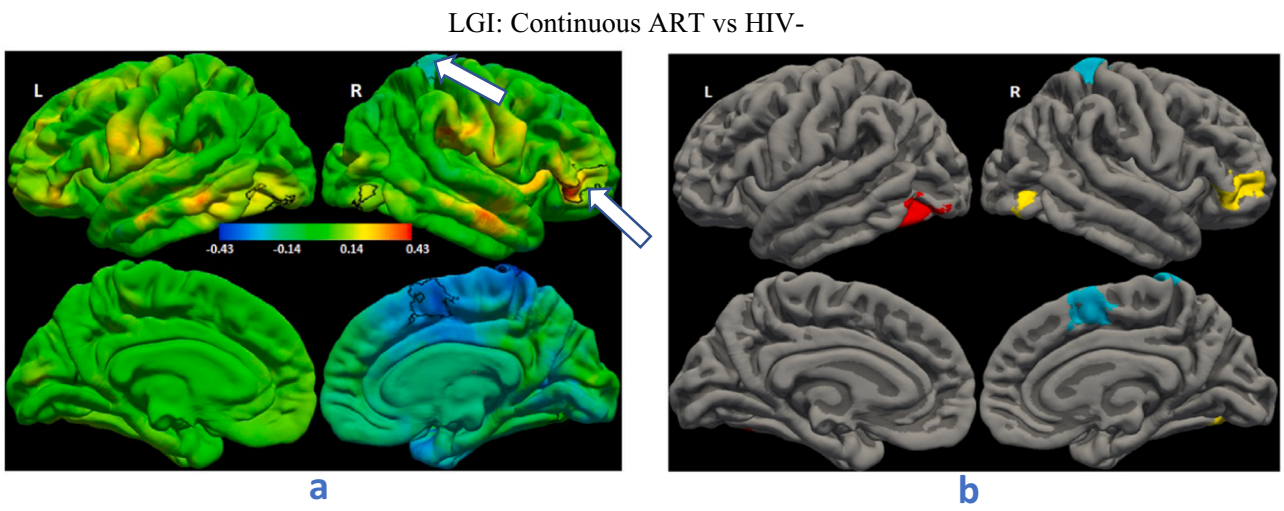

Fig. 4. Results from vertex-wise regression of local gyrification indices against group (Continuous ART vs HIV- controls), controlling for sex and age at scan. Images on the left are colour maps of unstandardized parameter estimates for group, and images on the right show only clusters where the groups differed significantly (cluster size corrected threshold of $p < 0.05$). Positive regression coefficients (red/yellow) indicate Continuous ART > controls (HIV-), and negative coefficients (cyan/blue) indicate Continuous ART < controls (HIV-). The colour bar scale indicates the effect size (Cohen's *d*) and applies to both lateral (top) and medial (bottom) views. Compared to HIV- controls, children receiving continuous ART demonstrated lower gyrification in right posterior superior frontal and superior parietal lobule (indicated with a white arrow) regions, but higher gyrification in left fusiform, right rostral middle frontal / pars triangularis / pars orbitalis (indicated with a white arrow) and lateral occipital regions.

Effect of ART interruption

As hypothesized, a short duration interruption have little effect on children's cortical thickness at this age – the only differences in cortical thickness due to interruption were thinner cortex in a small left lateral occipital region compared to continuous ART and thicker cortex in small left frontal and right insular regions compared to controls. In contrast, interruption effects on LGI were substantial. Children with ART interruption showed lower gyrification than both children on continuous ART and uninfected controls in a left precuneus/superior parietal region. When compared to controls, the continuous ART group showed gyrification alterations in different regions from those seen in the interrupted group, except that both groups demonstrated greater gyrification in a similar right lateral occipital region. Notwithstanding these findings, Ananworanich et al. (2016) reported no effect of interruption on neurocognitive measures 2 years after ART resumption in children whose ART was interrupted for 48 weeks or till CD4% dropped to 20% (Paediatric European Network for Treatment of AIDS (PENTA), 2010). A short duration ART interruption also did not affect immune health in children after resumption of treatment (Lewis et al., 2017; Wamalwa et al., 2016). Since CT in both frontal and insular regions decrease logarithmically from 1 to 6 years (Remer et al., 2017), disruptions to the normal age-related decrease during interruption could account for the thicker cortex seen at age 5 years. The small size of these regions suggests, however, that a short interruption period may have less severe consequences on children's cortical thickness if ART is initiated early.

Gyral folding appears affected to a greater extent by early childhood events and we have previously reported its long-term sensitivity to timing of ART initiation (Nwosu et al., 2018). This sensitivity may play a role in the differing effects on gyrification seen here in ART-interrupted and continuously treated children. Our finding of lower LGIs in right anterior cingulate in the children with ART interruption compared to controls corresponds with our previous finding at age 7, of lower medial frontal gyrification in children who initiated ART before 12 weeks, more than half of whom had ART interruption. In both studies, children in the interruption group all started ART before 12 weeks, while those starting ART after 12 weeks were on continuous treatment. Notably, interruption coincided with a period when rostral anterior cingulate gyrification, especially the right, increases rapidly (Remer et al., 2017). In addition, the right pars triangularis region where continuously treated children showed greater LGIs than controls at age 5 years, is adjacent to a region where later ART initiation was associated with increasing LGIs at age 7 years. These overlapping yet different results make it difficult to distinguish between treatment- and interruption-related effects. Since at age 5 only 12 children on continuous treatment started ART before 12 weeks, we had insufficient power to rule out a treatment timing effect. However, LGI differences between ART-interrupted and continuous treatment groups did persist in *posthoc* analyses controlling for age at ART initiation, as well as shorter cumulative duration of ART treatment.

Further, it should be noted that because of the design of the initial trial, children on continuous ART were generally older at ART initiation, and therefore experienced their nadir CD4% at a younger age (before starting treatment) than children who had ART interruption, who tended to have experienced a nadir CD4% during treatment interruption i.e. after 40 or 96 weeks of treatment. It is therefore ambiguous whether observed differences in cortical structure are related to interruption itself or an older age of severe immune compromise. Alternatively, earlier exposure to ART drugs may have a neurotoxic effect on cortical development (Shah et al., 2016; Robertson et al., 2012).

In typical development, LGIs in the right lateral occipital region decreases logarithmically by about 5% from 1–6 years (Remer et al., 2017). Greater LGIs in this region at age 5 years in both ART interrupted and continuously treated children, in whom viral load trajectories would have differed across the first few years of life, suggests therefore that changes seen at this age may be due to effects of HIV on early life gyral formation, before treatment differences would have played a role. Of note here is that we could not distinguish *in utero* from intrapartum infection, with the former associated with longer HIV exposure. Greater LGIs in a region characterized by age-related decreases, together with the absence of differences in this region at age 7 years (Nwosu et al., 2018), points to developmental delay. This region was probably not seen in the HIV+ group as a whole due to the fact that the regions showing differences in the two subgroups were in slightly different, albeit adjacent, locations.

From ages 1–6 years, gyrification changes in the left precuneus in typical development follow a quadratic trajectory – slightly decreasing first, followed by a slight increase of about 2.7% (Remer et al., 2017). Since the interruption period in the children studied here coincides with

the trajectory minimum, it is possible that higher viral loads during and following interruption disrupt the normal age-related LGI increase that should be occurring in this region at this time, resulting in lower LGIs at age 5 years.

While changes in right pars triangularis, superior frontal and superior parietal cortices in typically developing children from ages 1–6 years are small, making interpretation of LGI differences observed in these regions in continuously treated children difficult, LGI in left fusiform decreases logarithmically by 8.5% over this period (Remer et al., 2017). Greater LGIs in this region at age 5 years in only the continuously treated group, 52% of whom started ART after 12 weeks, therefore points to an effect of HIV on the normal age-related decrease that should have occurred in the first year of life.

Study limitations

A limitation of this study is the small sample size of the HIV+ subgroups (children on continuous and interrupted ART); a larger sample size might be better powered to reveal the extent of differences in cortical structure and would have allowed us to examine the potential role of HIV disease severity. Another limitation is that using a cluster forming threshold of $p < 0.05$ and the Monte Carlo simulation method to calculate cluster size thresholds, slightly elevates false positive rates for cortical thickness analyses. However, using a lower threshold will affect the statistical power of the results (Greve and Fischl, 2018). In this study, at a threshold of $p < 0.001$, none of the group comparisons yield any significant clusters.

Conclusion

Our study was focused on investigating the effects of early ART initiation and interruption on the brain morphometry of children at the neurodevelopmentally critical age of 5 years. Cortical folding (gyrification) is sensitive to early life events and accordingly we find it affected by ART interruption. In contrast, cortical thickness development at age 5 years is less affected by early life events. Immune health resilience in children can translate into long term preservation of neurodevelopment especially for those on early and continuous treatment. The neuropsychological implication of morphometric alterations in cortical folding requires investigation in future studies.

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