Altered brain morphometry in 7-year old HIV-infected children on early ART

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Abstract

Background—Even with the increased roll out of combination antiretroviral therapy (cART), paediatric HIV infection is associated with neurodevelopmental delays and neurocognitive deficits that may be accompanied by alterations in brain structure. Few neuroimaging studies have been done in children initiating ART before 2 years of age, and even fewer in children within the critical stage of brain development between 5-11 years. We hypothesized that early ART would limit HIV-related brain morphometric deficits at age 7.

Methods—Study participants were 7-year old HIV-infected (HIV+) children from the C̲hildren with H̲IV E̲arly A̲nti̲retroviral Therapy (CHER) trial whose viral loads were supressed at a young age, and age-matched uninfected controls. We used structural magnetic resonance imaging (MRI) and FreeSurfer (http://www.freesurfer.net/) software to investigate effects of HIV and age at ART initiation on cortical thickness, gyrification and regional brain volumes.

Results—HIV+ children showed reduced gyrification compared to controls in bilateral medial parietal regions, as well as reduced volumes of the right putamen, left hippocampus, and global white and gray matter and thicker cortex in small lateral occipital region. Earlier ART initiation was associated with lower gyrification and thicker cortex in medial frontal regions.

Conclusions—Although early ART appears to preserve cortical thickness and volumes of certain brain structures, HIV infection is nevertheless associated with reduced gyrification in the parietal cortex, and lower putamen and hippocampus volumes. Our results indicate that in early childhood gyrification is more sensitive than cortical thickness to timing of ART initiation. Future work will clarify the implications of these morphometric effects for neuropsychological function.

Keywords

gyrification; morphometry; CHER; neurodevelopment; cortical thickness; paediatric HIV

1. Introduction

With increased antiretroviral therapy (ART) roll-out, the mortality rate of perinatally HIV-infected (HIV+) children has decreased substantially (Freeman 2016; Cotton *et al*. 2013; Violari *et al*. 2008). Hence, pediatric HIV is now a chronic infection and research efforts have shifted to understanding the effects of HIV and various combination ART regimens during childhood development (Freeman 2016; Blokhuis *et al*. 2016).

Even in the ART era, pediatric HIV infection is associated with neurodevelopmental delay and neurocognitive deficits such as language impairment, psychomotor slowing, and processing speed delay (Donald *et al*. 2014; Wilmhurst *et al*. 2014; Foster *et al*. 2006; Koekkoek *et al*. 2008; Martin *et al*. 2006; Van Arnhem *et al*. 2013). Behavioural abnormalities include lack of concentration and attentiveness, hyperactivity, anxiety and conduct disorders (Musielak and Fine 2015; Govender *et al*. 2011). Neurodevelopmental delays and deficits may be exacerbated by environmental and physical early life stressors, such as prenatal exposure to alcohol or drugs, and trauma, all of which are prevalent in South Africa (Kader *et al*. 2012).

Neuroimaging studies show that even when ART is initiated in childhood, there are alterations in brain structure due to HIV and/or ART (for reviews see Musielak and Fine 2015; Hoare *et al*. 2014). Findings have included lower cortical and total gray matter (GM) volumes (Lewis-de los Angeles *et al*. 2017; Cohen *et al*. 2016); regional cortical (Lewis-de los Angeles *et al*. 2017; Sarma *et al*. 2014) and subcortical (Yadav *et al*. 2017; Lewis-de los Angeles *et al*. 2016) volume and shape alterations; ventricular enlargement and sulcal widening (Van Arnhem *et al*. 2013); regional cortical thickness increases and decreases (Yadav *et al*. 2017); global (Cohen *et al*. 2016) and local white matter (WM) atrophy (Sarma *et al*. 2014); WM lesions/abnormalities (Hoare *et al*. 2015; Ackermann *et al*. 2016, 2014; Uban *et al*. 2015; Van Arnhem *et al*. 2013), and basal ganglia calcification (Govender *et al*. 2011).

The Children with HIV Early Antiretroviral Therapy (CHER) clinical trial (Violari *et al*. 2008; Cotton *et al*. 2013) showed that early HIV diagnosis and ART initiation (6-12 weeks of age) reduced infant mortality and HIV progression compared to standard 2006 guidelines which advised initiating ART when the percentage of CD4+ T lymphocytes (CD4 percentage) - an indicator of immune system health -declined below 25% or for severe clinical disease (WHO, 2006). Since 2008, most guidelines recommend initiating ART as

soon as possible for all HIV+ infants regardless of CD4 measures, even if asymptomatic (WHO, 2008; PENTA 2009; ART Guideline, 2008).

Although early ART initiation improves clinical outcomes for HIV+ children (Phongsamart *et al.* 2014; Crowell *et al.* 2015; Laughton *et al.* 2013; Brahmbhatt *et al.* 2014), ART alone cannot completely reverse the neurological effects of HIV (Whitehead *et al.* 2014; Lowenthal *et al.* 2014; Van Arnhem *et al.* 2013; Laughton *et al.* 2013; Mbugua *et al.* 2016; Ackermann *et al.* 2016). In addition, there is concern about potential adverse effects of ART including metabolic abnormalities (Vigano *et al.* 2010) and neurotoxicity (Robertson *et al.* 2012).

Few neuroimaging studies have been done in children initiating ART before 2 years of age, and even fewer in children within the critical stage of brain development between 5-11 years. Most studies of HIV+ children in this age range focused on neurobehavioral and cognitive parameters (Govender *et al.* 2011; Shanbhag *et al.* 2005; Martin *et al.* 2006), while the few existing neuroimaging studies included children over a wide age range, with later ART initiation and/or without details of viral suppression (Van Arnhem *et al.* 2013; Van Dalen *et al.* 2016; Yadav *et al.* 2017). These studies may not be relevant to the growing population of children on early ART in whom viral loads are suppressed from a young age.

Despite early ART initiation and viral load suppression, HIV+ children from the CHER cohort demonstrate basal ganglia metabolite level and white matter alterations at age 5 years (Mbugua *et al.* 2016; Ackermann *et al.* 2016). Morphometric brain development of these children is unknown. Here, we used FreeSurfer's automated cortical and subcortical analysis methods to investigate the effects of HIV and timing of ART initiation on brain morphometry in a neuroimaging follow-up study of the Cape Town CHER cohort at age 7 years. To our knowledge, this is the first study of brain morphometry in a homogeneous sample of children in a narrow age range (7.00 – 7.83 years) all of whom initiated ART before 76 weeks and 93% of whom had viral load suppression at time of scanning.

We hypothesized that early ART would prevent much of the neurological damage associated with perinatal HIV infection and would lead to no or small differences from the uninfected control group with respect to gyrification, cortical thickness and gray and white matter volumes. Further, since cortical gyrification is positively related to cognitive performance and cortical volume, but negatively related to cortical thickness in many regions of the cortex (Gautam et al. 2015), we hypothesized that younger age of ART initiation would be associated with greater gyrification, thinner cortex, and larger brain volumes.

2. Methods

Study participants

The study population comprised 103 Xhosa children (sixty-one HIV+, forty-two uninfected controls, age 7.21 ± 0.14 years) from a follow-up study of CHER trial participants, conducted at the Family Clinical Research Unit at Tygerberg Children's Hospital, Cape Town. On the CHER trial, HIV+ infants with CD4 percentage 25% were randomized to limited ART between 6 and 12 weeks of age for either 40 or 96 weeks and restarted when

clinical and/or immunological criteria were met, or started ART only when they became symptomatic or CD4 percentage dropped below 20% (25% in the first year), as per guidelines at the time (WHO, 2006). Of the HIV+ children, forty-six started ART at or before 12 weeks of age (Before-12wk) and fifteen after 12 weeks of age (After-12wk). All HIV+ children had started ART by 76 weeks of age and received comprehensive immunological and clinical follow-up thereafter. First line ART regimen consisted of Zidovudine (ZDV) + Lamivudine (3TC) + Lopinavir-Ritonavir (LPV/r, Kaletra®) (CHER, 2010; Violari *et al.* 2008; Cotton *et al.* 2013). Viral loads were suppressed (<400 copies/mL) in 93% of HIV+ children at the time of scanning. The uninfected control group was recruited from an interlinking vaccine trial (Madhi et al., 2010) and included both children born to HIV+ mothers (HIV-exposed, uninfected; HEU; n=20) and to uninfected mothers (HIV-unexposed; HU; n=22).

Study participants' cognitive abilities were assessed on the Kaufman Assessment Battery for Children second edition (KABC-II) at 7 years as part of a longitudinal neurocognitive study (Kaufman & Kaufman, 2004). The Luria model of the KABC-II is considered culture fair and appropriate for use within the South African context (Boivin and Giordani, 2009; Jansen and Greenop, 2008; Skuy et al., 2000; Van Wyhe et al., 2017). Here we provide two summary measures from the KABC-II, namely the Mental Processing Index (MPI), which is a composite score of intellectual functioning from four subscales (Learning, Planning, Sequential processing and Simultaneous processing), and the Non-verbal Index (NVI), a composite score obtained from subtests requiring non-verbal reasoning. Test scores are interpreted relative to standardized norms with mean 100 and SD 15; MPI and NVI standard scores between 85 and 115 are considered average.

Image acquisition and analysis

All participants received a T1-weighted high-resolution structural MRI scan on a 3T Allegra scanner (Siemens, Erlangen, Germany) at the Cape Universities Brain Imaging Centre (CUBIC). Children were scanned in sagittal orientation using a 3D echo planar imaging (EPI) navigated (Tisdall *et al.* 2012) multiecho magnetisation prepared rapid gradient echo (MEMPRAGE; van der Kouwe *et al.* 2008) sequence (FOV 224 × 224 mm^2, TR 2530 ms, TI 1160 ms, TE's = 1.53/3.19/4.86/6.53 ms, bandwidth 650Hz/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.

FreeSurfer version 6.0 (https://surfer.nmr.mgh.harvard.edu) was used for automated cortical reconstruction and to measure cortical thickness and local gyrification index (LGI) across the cortical surface, as well as regional and total brain volumes. FreeSurfer outputs were manually checked for errors in cortical and sub-cortical segmentations. Minor pial edits (skull strip corrections) were required for some of the outputs, but no white matter correction. There were no failures in the LGI computation. Subjects were excluded from

particular analyses if their mean values on these measures were extreme outliers (removed from the median of the sample by more than 3 times the interquartile range (IQR)).

Volumetric Analysis

Volumes of regions previously implicated in HIV infection, including the bilateral caudate, thalamus, hippocampus, putamen and lateral ventricles, as well as the corpus callosum, global white matter (WM), global gray matter (GM), and total intracranial volume (TIV), were analysed using the statistical analysis programming language, R (https://www.r-project.org/).

Linear regression was used to compare volumes between HIV+ and uninfected children. Since sex-related differences in cortical and sub-cortical structures have been reported in brain development (Fisher *et al.* 2016; Luder *et al.* 2010), sex was controlled for in all analyses. Analyses were repeated adjusting for TIV to examine whether regional volume changes were over and above the effect of HIV on TIV. Among HIV+ children, relationships of age at ART initiation and cumulative ART duration to total and regional brain volumes were also investigated adjusting for the effect of duration of ART interruption. Since treatment interruption could expose children's brains to HIV, this may have negated some of the benefits of earlier ART. Duration of interruption was set to zero for children on continuous treatment. We report *p*-values both uncorrected and after adjustment for multiple comparisons using the false discovery rate (FDR) method (Benjamini *et al.* 1995).

Vertex-wise analysis of cortical thickness and LGI

Cortical thickness and LGI were compared between HIV+ and uninfected children vertex-wise over the whole brain using the QDEC tool in FreeSurfer. The interaction of sex and HIV infection status was also investigated. To investigate the possible effect of earlier ART initiation, we explored among HIV+ children the relationship between age at ART initiation and morphometric measures, controlling for duration of ART interruption, as well as differences between the Before-12wk and After-12wk groups. To examine whether reduced gyrification in the Before-12wk group was attributed to treatment interruption in some children, we also compared the After-12wk group separately to children in the Before-12wk group who were interrupted and those who were not. Further, vertex-wise comparisons were performed between Before-12wk and After-12wk children and uninfected controls separately. The smoothing kernel for cortical thickness analyses was 10mm FWHM and no smoothing was used for LGI. Results of vertex-wise analyses controlling for sex were thresholded at an initial uncorrected threshold of $p<0.05$. Because of the spatial smoothness of the data, uncorrected p-values at neighbouring vertices can be assumed to covary to some degree, forming clusters. To adjust for multiple comparisons across vertices, cluster size limits for a two-tailed test, threshold of $p<0.05$, and for data of similar smoothness, were generated using Monte Carlo simulation (Forman et al., 1995). We report clusters surviving a cluster-size corrected threshold of $p<0.05$, with no adjustment for the two hemispheres.

3. Results

Sample characteristics

Only one child, a girl in the Before-12wk group, was excluded due to a poor quality MRI. Her data on a number of measures were also extreme outliers (> 3 IQRs below the 25th percentile). We therefore present data for 102 children. Population characteristics for all subjects are presented in Table 1. Groups did not differ in age, sex, TIV and MPI and NVI on the KABC-II (administered at age 7 years). Table 2 presents sample characteristics and clinical measures for HIV+ children in the Before-12wk and After-12wk groups. Since children were randomised to treatment arms, groups do not differ on any demographic or disease-related variables, except for CD4 count at age 7, which we controlled for in all analyses involving comparisons between the Before-12wk and After-12wk groups. Notably, no child starting ART after 12 weeks had treatment interrupted whereas the Before-12wk group included 28 children (62%) whose treatment was interrupted. All except 5 children had undetectable viral loads (<400 copies/mL) by 2 years of age, and all except 4 at the time of scanning.

Volumetrics

After controlling for TIV, volumes of global gray and white matter, bilateral hippocampus and putamen, and right thalamus were smaller (p 0.05) in HIV+ children compared to controls (Table 3). On adjusting for multiple comparisons, global gray and white matter, left hippocampus and right putamen volumes remained significantly smaller, while right hippocampus, left putamen and right thalamus showed a trend for being smaller in HIV+ children than uninfected controls. Age at ART initiation and cumulative duration on ART showed no relationship with both global and regional brain volumes after multiple comparison correction.

Vertex-wise analysis of cortical thickness and LGIs

HIV+ children demonstrated thicker cortex compared to uninfected controls in a small left inferior lateral occipital region (MNI co-ordinates at peak: -32.9, -83.4, 1.6, size: 865.57 mm^2) (Figure 1), and lower local gyrification in bilateral paracentral (Left: MNI co-ordinates at peak: -6.4, -35.8, 56.5, 1966.90 mm^2; Right: MNI co-ordinates at peak: 11.2, 1.4, 46.1, 1109.49 mm^2) and right temporal (MNI co-ordinates at peak: 54.6, -12.3, -17.3, 802.37 mm^2) regions (Figure 2).

Although there was no interaction effect of sex on the relationship between HIV status and cortical thickness significant at p<0.05, there was a significant interaction effect of sex on the relationship between HIV status and LGI in bilateral parietal/middle temporal regions (left: MNI co-ordinates at peak: -47.2, -60.0, 8.8, 2538.16 mm^2; Right: MNI co-ordinates at peak: 45.6, -52.0, 19.7, 2484.04 mm^2) (Figure 3a). The plots in Figure 3b show that the mean LGIs in the bilateral clusters were similar or higher in HIV+ girls compared to uninfected girls (Left p=0.09, Right: p=0.004), while LGIs of HIV+ boys were lower than in uninfected boys (Left p=0.01, Right p=0.02).

Linear regression that adjusted for sex and duration of interruption showed that right medio-frontal (MNI co-ordinates at peak: 10.3, 51.2, 3.3, cluster size: 882.87 mm^2) cortex was thinner with later ART initiation (Figure 4a), while LGI was greater with later ART initiation in symmetric bilateral medial frontal regions (Left: MNI co-ordinates at peak: -5.7 29.6 13.6, cluster size: 3609.06 mm^2; Right: MNI co-ordinates at peak: 14.7, 39.0, 9.2, cluster size: 5285.53 mm^2), as well as a right lateral fronto-temporal region (MNI co-ordinates at peak: 43.2, -0.8, -18.7, cluster size: 838.81 mm^2) (Figure 4b). Group analyses adjusted for sex confirmed that After-12wk children had significantly greater LGIs than both interrupted and continuous Before-12wk children in the same bilateral medial frontal regions. Comparison with the uninfected children showed that whereas the After-12wk children had greater LGIs than the uninfected children in these bilateral frontal regions, the Before-12wk children had smaller LGIs in similar regions.

4. Discussion

This is the first study reporting possible effects of HIV and timing of ART initiation on cortical thickness and gyrification in children. Specifically, HIV+ children demonstrated thicker cortex in a small left inferior lateral occipital region and lower gyrification in bilateral paracentral and right temporal regions than uninfected controls, despite early ART and viral load suppression. Additional HIV-related gyrification reductions were evident in lateral parieto-temporal regions in boys, but not in girls. Contrary to our hypothesis, ART initiation at a younger age was associated with less gyrification in bilateral medial frontal and right fronto-temporal regions, but thicker cortex in an overlapping right medial frontal region. Separate analyses demonstrated greater gyrification in similar bilateral medial frontal regions in After-12wk children compared to both uninfected controls and Before-12wk children, who had the least gyrification in these regions. WM and GM volumes, right putamen and left hippocampus were relatively smaller in HIV+ children after adjustment for reductions in total brain volume and multiple comparisons, while timing of ART initiation did not affect global or regional brain volumes.

Volumetrics

Our finding of lower global white and gray matter volumes is consistent with other studies in youths on ART with viral load suppression (Cohen *et al.* 2016; Lewis-de los Angeles *et al.* 2017). However, findings in subcortical regions have been less consistent. While Lewis-de los Angeles *et al.* (2016) found subcortical shape deformations in the absence of HIV-associated volumetric differences, Yadav and colleagues (2017) reported hippocampal volume decreases and nucleus accumbens increases in slightly older HIV+ children, and Blokhuis *et al.* (2017) reported trend-level but not significant putamen increases.

Before the roll-out of early ART, common structural neuroimaging findings in HIV+ children included ventricular enlargement, white matter signal abnormalities, cerebral and subcortical atrophy, corpus callosum damage and basal ganglia calcification (George *et al.* 2009; Hoare *et al.* 2014, Martin *et al.* 2006). The subcortical neuronal atrophy observed in HIV infection was thought to be due to the proximity to the ventricle, which acts as a channel for cerebrospinal fluid and a carrier of HIV. This proximity may facilitate easier

penetration by HIV-infected mononuclear cells and also HIV toxic products, such as gp120 and Tat, leading to neuronal atrophy (Ances *et al.* 2012). Early ART has both clinical and immunological benefits by suppressing HIV replication, in the CNS and elsewhere, and restoring immune function, thereby protecting brain morphology and neurodevelopment (Laughton *et al.* 2012; Cotton *et al.* 2013; Lindsey *et al.* 2007; Violari *et al.* 2008). Notably, we did not find volume reduction in the caudate and a trend in right thalamus – the regions closest to the ventricles.

The decreased volume observed here in the putamen and hippocampus, despite early ART initiation and viral load suppression, suggests either ongoing neurodevelopmental delay or damage from early neuro-infiltration that persists into childhood. The latter ties in with recent spectroscopy findings in the same cohort where immunocompromise at baseline was associated with lower basal ganglia metabolite levels (choline, NAA) five years later (Mbugua *et al.* 2016).

Interestingly, we did not find corpus callosum (CC) reductions or ventricular enlargement. Although CC atrophy was found in youths with suppressed viral loads some of whom started ART early (Sarma *et al.* 2014; Yadav *et al.* 2017), most children included in studies reporting ventricular enlargement did not have suppressed viral loads (Hoare *et al.* 2014; van Arnhem *et al.* 2013; Martin *et al.* 2006), whereas viral loads were suppressed in 93% of children in this study. It is possible that CC atrophy is not evident at age 7 years, as children in the two studies cited above were older and initiated ART over a wider age range, whereas in our study all children initiated ART before 76 weeks of age. These data suggest that earlier ART may be neuroprotective to the CC, consistent with results of recent DTI findings in our cohort where no microstructural differences were found between HIV+ and control children in the CC at age 5 years (Ackermann *et al.* 2016). However, manual segmentation in these children at 5 years showed corpus callosum reductions in HIV+ children, even though automated segmentation showed little difference between groups (Randall et al., 2017). CC volume alterations may be so small that automated segmentation measures used here are not sensitive enough to detect differences, due to imperfect co-registration of pediatric brains at this age to adult templates (Narayanan *et al.* 2016; Yoon *et al.* 2009). A study by Andronikou *et al.* (2015) including HIV+ children from our cohort (age range 7 to 49 months) and using semi-automated segmentation methods, also did not find CC volume and thickness differences compared to controls.

LGI and cortical thickness

Contrary to our hypothesis that early ART would minimise morphometric alterations, HIV+ children had lower gyrification than uninfected controls in large bilateral medial parietal and right temporal regions, with affected regions being more extensive in boys than in girls. Although sulcal widening, characterised by reduced gyrification, curvature and steepness (White *et al.* 2010), was reported in HIV+ children on ART (Van Arnhem *et al.* 2013), only 24% of children in that study had suppressed viral loads compared to 93% here. Unfortunately, that study reported only the incidence of sulcal widening and not the locations, making direct comparison of affected regions impossible. Sex differences are not

surprising as both gyrification and cortical thickness differ between sexes (Fisher *et al.* 2016; Luder *et al.* 2010; Im *et al.* 2006).

The fact that HIV+ children had thicker cortex than controls only in one small left lateral occipital region suggests that early ART may have reversed or prevented some of the previously reported effects of HIV on the cortex. This is consistent with reports of improved neurodevelopmental function in HIV+ children started on ART in early infancy (Brahmbhatt *et al.* 2014; Cotton *et al.* 2013; Crowell *et al.* 2015; Laughton *et al.* 2012; Lindsey *et al.* 2007; Shanbhag *et al.* 2005). However, a recent study by Yadav and colleagues (2017) in 10 to 11-year old HIV+ children on ART found regional cortical thickness decreases in bilateral postcentral and right superior temporal regions, but *increases* in bilateral medial frontal regions, which the authors suggest could be related to stress-induced hypertrophy of medium spiny neurons. It is noteworthy that the regions showing greater cortical thickness in that study overlap with those where we found lower gyrification and thicker cortex in children who started ART before 12 weeks of age.

Effects of timing of ART initiation

Previous studies have shown a relationship between earlier ART initiation and improved neurodevelopmental (IQ, memory, psychomotor, and behavior) outcomes, at least in the short term (Brahmbhatt *et al.* 2014; Crowell *et al.* 2015; Laughton *et al.* 2012), as well as metabolic advantages in the basal ganglia at age 5 years (Mbugua *et al.* 2016). Although we found no effect of age of ART initiation on brain volumes, the relationships observed in the medial frontal regions for both cortica thickness and gyrification, and for gyrification in fronto-temporal regions, suggest that time of ART initiation affects cortical development in these regions. The fact that more extensive regions show greater gyrification with later ART initiation than thinner cortex, suggests that gyral formation in early childhood may be more sensitive to earlier ART than cortical thickness. It is known that gyrification is influenced primarily by early events, in contrast to cortical thickness, which is most affected by maturational changes during childhood, adolescence and early adulthood (Schaer *et al.* 2012).

The findings of positive association between bilateral frontal LGI and age at ART initiation, and reduced frontal LGI in Before-12wk children compared to both controls and After-12wk children, suggest that earlier ART rather than HIV infection reduces gyrification in this region. In the region showing an effect of ART initiation age on cortical thickness, Before-12-wk children, who had lowest gyrification, also had the thickest cortex (2.88 ± 0.19 mm); thicker than both controls (2.78 ± 0.19 mm, *p*=0.005) and After-12wk children (2.70 ± 0.15 mm, *p*<0.001). ZDV, used in first line treatment, has been associated with mitochondrial dysfunction (Robertson *et al.* 2012; Musielak & Fine, 2015) and neurological abnormalities (Tardieu *et al.* 2005). A DTI study in children from the same cohort at age 5 years similarly found poorer regional white matter (WM) integrity in children starting ART before 12 weeks compared to those starting after 12 weeks (Ackermann *et al.* 2016). In that study, the poorer WM integrity was attributable to treatment interruption. In contrast, the relations of LGI and cortical thickness with age at ART initiation in our study survived adjustment for treatment interruption. Also, gyrification increases in the After-12wk children

were evident compared to both interrupted and continuous Before-12wk children separately, confirming that the lower gyrification observed here in the Before-12wk group is not a consequence of treatment interruption.

It is possible that higher gyrification in After-12wk children is a consequence of abnormally increased sulcal depth in children due to untreated HIV infection. Abnormal increases in gyrification relative to healthy controls are observed in children and adults, for example, in autism (Wallace *et al.* 2013), Williams syndrome (Gaser *et al.* 2006), schizophrenia (Schultz *et al.* 2010) and preterm birth (Kesler *et al.* 2006), and together with thinner cortex in children with developmental dyslexia (Williams et al. 2017). In this scenario, unless earlier ART and untreated HIV have opposite effects on early gyrification, it is difficult to explain why children receiving ART before 12 weeks should have lower frontal LGIs than controls while children receiving ART after 12 weeks have higher LGIs than controls in similar regions. It should be noted, however, that the control group includes HEU children, exposed in utero to HIV and antiretrovirals which could have influenced brain development, and HU children (Robertson *et al.* 2012; Le Doare *et al.* 2012). Even though HEU and HU children did not differ on any demographic variables, when comparing the After-12wk children to HEU and HU children separately, the After-12wk children demonstrated greater LGIs in the same bilateral frontal regions than HEU but not HU children. These results suggest that gyrification increases in the After-12wk children may be attributable to lower gyrification in HEU children. Notably, cortical thickness in the region showing association with age at ART initiation did not differ between After-12wk and uninfected children (p=0.16) Neuropsychological test data are needed to understand the implication of increased gyrification in these regions in After-12wk children and to reveal whether earlier ART affects cognitive function in the longer term.

Our study includes a relatively large sample size, at a specific age, with participants on homogenous ART regimens and clinical follow-up from birth until age 7 years. Although the narrow age range is a strength of the study, it limits our ability to draw any conclusions about longitudinal brain development.

In this study, we could not disentangle the contributions of HIV and ART to the current findings, due to all children being on ART, although associations with ART initiation age may provide some insights. This is, however, complicated by the fact that a subset of the children receiving early ART had protocol-defined treatment interruption while those starting later remained on continuous ART. Although we controlled for duration of treatment interruption, it is possible that there are critical times in which exposure to HIV may impair gyrification or cortical thickness. In addition, environmental variables associated with HIV may also profoundly affect neurodevelopment. Even though the uninfected control children were from a similar disadvantaged socioeconomic environment, HIV+ children are more likely to have experienced stressors such as poor health, nutrition or the loss of a parent, which may influence brain development. The limited socioeconomic and environmental data obtained for these children makes it difficult to explore these factors fully in the current study. Future studies obtaining more descriptive sociodemographic and environmental data may be able to untangle the effects of these variables from the effects of HIV/ART itself on

brain development. The co-registration of children's brains to an adult brain template is also a limitation and may have obscured some morphometric outcomes.

5. Conclusions

In this study regional differences in cortical thickness, gyrification, global white and gray matter volume, left hippocampus and right putamen suggest persisting and residual structural alterations at age 7 years despite early ART initiation and viral load suppression. However, no differences in volumes of many subcortical regions, corpus callosum, and lateral ventricles were found between HIV+ children and controls. There was also no effect of earlier ART on brain volumes at this age, but decreased medial frontal gyrification and increased cortical thickness in children starting ART before 12 weeks relative to both controls and children initiating ART after 12 weeks. Neuropsychological scores may clarify whether these frontal cortical changes are associated with better or worse cognitive outcomes.

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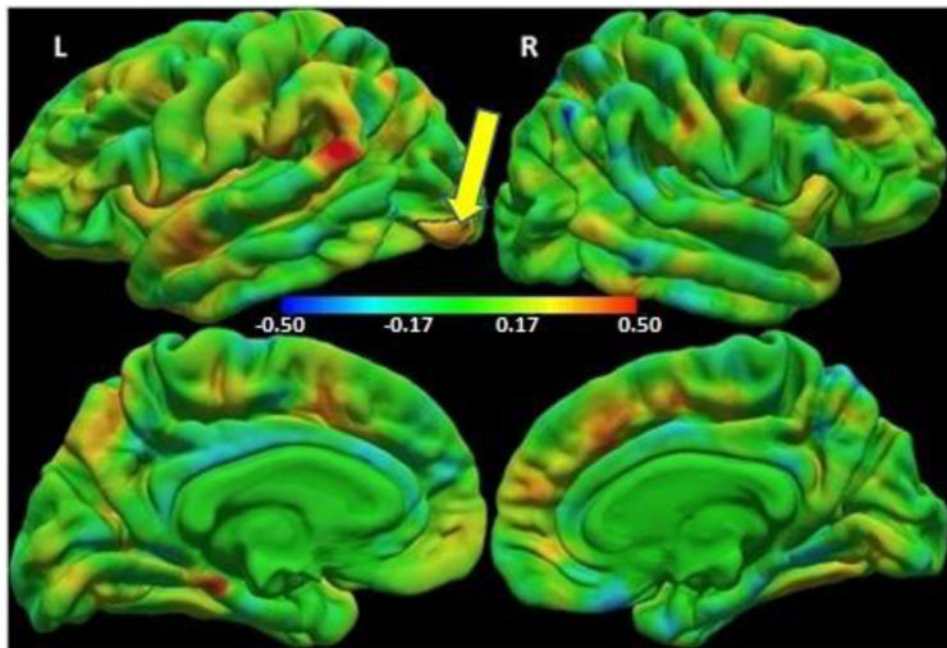
Fig. 1.
Colour map of regression coefficients for cortical thickness (mm) against group (HIV+ children/controls) controlling for sex. Positive regression coefficients (red/yellow) indicate HIV+ > controls, and negative coefficients (cyan/blue) indicate HIV+ < controls. The color bar scale applies to both lateral (top) and medial (bottom) views.

The small left lateral occipital region outlined in black shows where HIV+ children have thicker cortex compared to uninfected controls at a threshold of $p<0.05$, with a cluster size corrected threshold of $p<0.05$ (Peak MNI co-ordinates 32.9, -83.4, 1.6, size 865.57 mm^2).
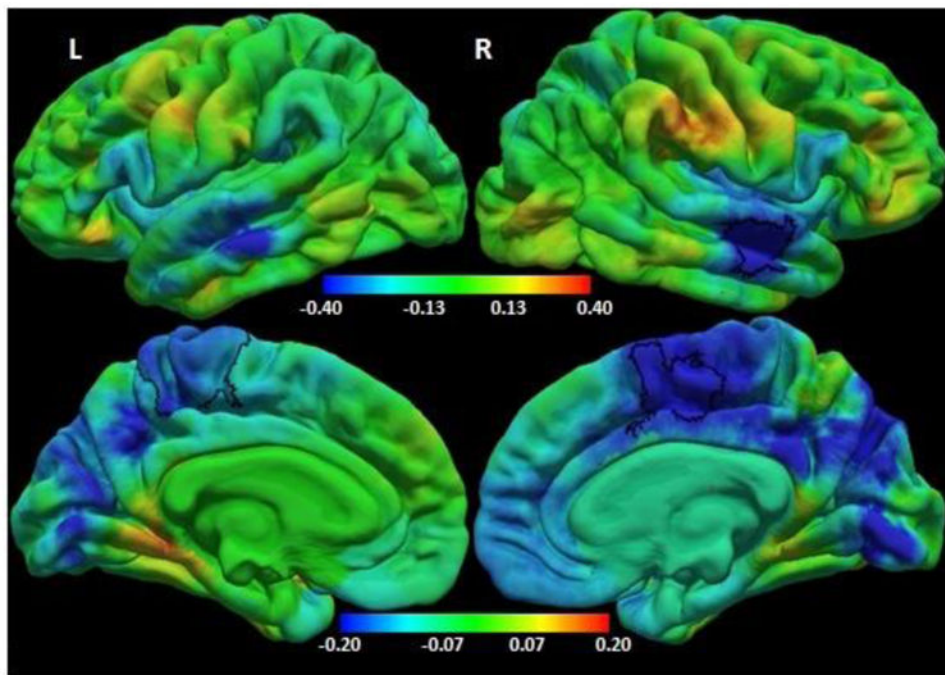
Fig.2.
Colour map of regression coefficients for local gyrification index (LGI) against group (HIV+ children /controls) controlling for sex. Positive regression coefficients (red/yellow) indicate HIV+ > controls, and negative coefficients (cyan/blue) indicate HIV+ < controls. Note different color bar scales for lateral (top) and medial (bottom) views.

The bilateral paracentral (left: precentral/ postcentral/ paracentral/ posterior cingulate/ precuneus/ superior parietal gyri, MNI co-ordinates -6.4, -35.8, 56.5, size 1966.90mm^2; right: superior frontal/ paracentral/ posterior cingulate gyri, MNI co-ordinates 11.2, 1.4, 46.1, size 1109.49 mm^2) and right temporal (superior / middle temporal gyri, MNI co-ordinates 54.6, -12.3, -17.3, size 802.37mm^2) regions outlined in black show where HIV+ children have smaller local gyrification indices compared to uninfected controls at a threshold of $p<0.05$, with a cluster size corrected threshold of $p<0.05$.
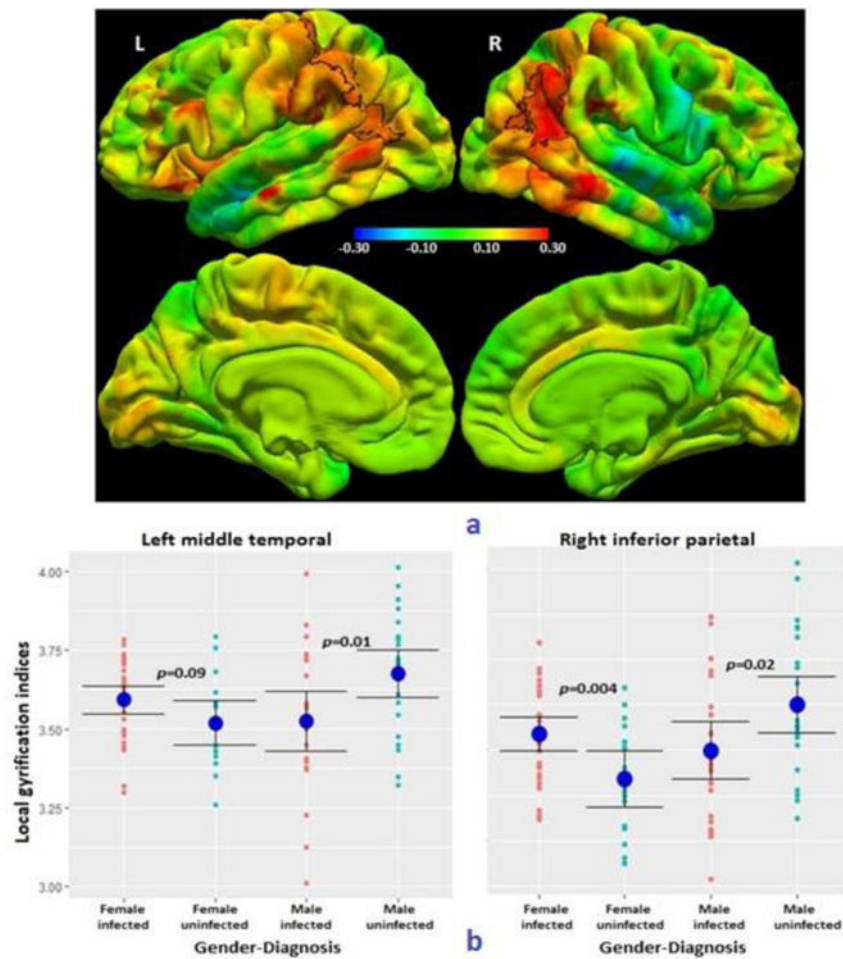
Fig. 3.

a) Colour map of regression coefficients for the interaction effect of sex on the relationship between HIV infection status and local gyrification index (LGI). The same color bar scale applies to all views.

The bilateral parieto-temporal regions outlined in black show a significant sex-HIV interaction effect at a threshold of $p<0.05$, with a cluster size threshold of $p<0.05$. (Left postcentral/ superior parietal/ supramarginal/ inferior parietal/ middle temporal gyri, MNI co-ordinates -47.2, -60.0, 8.8, size 2538.16 mm^2; right inferior parietal/ middle temporal/ supramarginal/ superior parietal gyri, MNI co-ordinates 45.6, -52.0, 19.7, size 2484.04 mm^2)

b) Plots of peak LGI (and group means ± SD) in the left and right parieto-temporal clusters, showing how the effect of HIV infection on LGIs differs in girls and boys. On the left, the peak LGI was located in the middle temporal gyrus, and on the right in the inferior parietal gyrus.
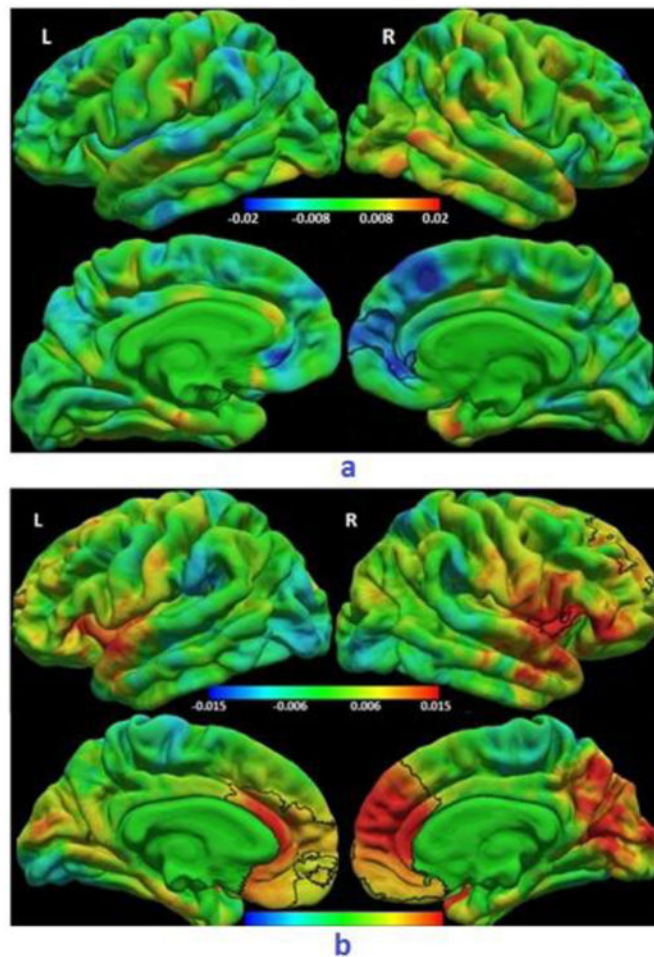
Fig. 4.
a) Colour map of regression coefficients for the regression of cortical thickness (mm) on age at ART initiation (weeks). The cluster outlined in black shows a right medial frontal region where older age at ART initiation was associated with thinner cortex at a threshold of $p<0.05$, with a cluster size threshold of $p<0.05$, after controlling for sex and duration of interruption. (Right rostral anterior cingulate/ medial orbitofrontal/ superior frontal/ frontal pole/ rostral middle frontal gyri, MNI co-ordinates 10.3, 51.2, 3.3, size 882.87 mm^2). A similar region showed thinner cortex in After-12wk children compared to Before-12wk children. The same color bar scale applies to all views.

b) Colour map of regression coefficients for LGI on age at ART initiation (weeks). Clusters outlined in black show (top) right lateral fronto-temporal (right pars orbitalis/ pars triangularis/ lateral orbitofrontal/ insula/ superior temporal, MNI co-ordinates 43.2, -0.8, -18.7, size 838.81 mm^2) and (bottom) bilateral medial frontal (left: medial orbitofrontal/ rostral anterior cingulate/ caudal anterior cingulate/ superior frontal/ frontal pole/ rostral middle frontal, MNI co-ordinates -5.7, 29.6, 13.6, size 3609.06 mm^2; right: caudal anterior cingulate/ rostral anterior cingulate/ medial orbitofrontal/ superior frontal/ rostral middle frontal/ frontal pole, MNI co-ordinates 14.7, 39.0, 9.2, size 5285.53 mm^2) regions where older age at ART initiation was associated with greater LGIs at a threshold of $p<0.05$, with a cluster size threshold of $p<0.05$, after controlling for sex and duration of interruption.

Similar regions showed greater gyrification in After-12wk children compared to Before-12wk children. Note different color bar scales for lateral (top) and medial (bottom) views.

Table 1

Sample characteristics

	HIV+	Uninfected	t or χ^2	p-value
Sample size (N)	60	42		
Age at scan (years)	7.20 (0.13)	7.23 (0.16)	-1.12	0.26
Number of males (% males)	28 (47%)	25 (58%)	1.47	0.25
Total intracranial volume (cm^3)	1427.99 (104.99)	1477.77 (130.77)	-1.43	0.16
Birth weight (g)	3079.91 (443.02)	3100.86 (466.99)	-0.23	0.82
Kaufman Assessment Battery for Children (KABC) Second edition composite scores:				
Mental processing index	72.18 (7.89)	73.13 (9.48)	-0.53	0.59
Non-verbal index	73.12 (10.85)	72.54 (11.21)	0.26	0.80

Values are N (% of total) or mean (standard deviation)

Table 2

Sample characteristics and clinical profiles of HIV+ children initiating ART before and after 12 weeks

	HIV+children			
	After-12wk (> 12 weeks)	Before-12wk (12 weeks)	t or χ^2	p-value
Demographics				
Sample size (N)	15	45		
Age at scan (years)	7.22 (0.10)	7.19 (0.14)	0.64	0.53
Males (% males)	5 (33%)	22 (49%)	0.68	0.41
Total intracranial volume (cm^3)	1295 (91.42)	1259 (108.77)	1.17	0.24
Birth weight (g)	3019.67 (439.05)	3087.39 (466.34)	-0.50	0.62
Clinical data at baseline (6-8 weeks)				
CD4 count (cell/mm^3)[a]	2010.60 (682.69)	1862.16 (992.67)	0.54	0.59
CD4% (cells/mm^3)	35.45 (7.78)	32.76 (10.04)	0.95	0.35
CD4/CD8[a]	1.32 (0.70)	1.30 (0.80)	0.09	0.93
CD8 count (cells/mm^3)	1888.50 (974.78)	1682.78 (949.77)	0.70	0.48
Viral load at enrolment				
High (>750,000 copies/mL)	9 (60%)	24 (53.3%)		
Low (400-750,000 copies/mL)	6 (40%)	21 (46.7%)		
Suppressed (<400 copies/mL)	0	0		
Clinical data at scan (7 years)				
CD4 count (cells/mm^3)	1446.27 (487.79)	1172.81 (451.39)	1.98	0.05
CD4% (cells/mm^3)	37.94 (6.20)	36.74 (6.64)	0.61	0.54
Viral load at scan (Age 7 years)				
High (>750,000 copies/mL)	0	0		
Low (400-750,000 copies/mL)	0	4 (8.9%)		
Suppressed (<400 copies/mL)	15 (100%)	41 (91.1%)		
Other				
Age at ART initiation (weeks)	34.14(17.56)	8.41(1.59)	9.99	<0.0001
Age at ART interruption (weeks)	-	63.77 (29.32)[b]	-	-
Age of first viral load suppression (weeks)[c]	47.57 (36.71)	33.50 (16.32)	1.06	0.29
Duration of ART interruption (weeks)	-	62.62 (76.08)[b]	-	-
Cumulative duration on ART (weeks)	342.70 (18.87)	318.70 (78.08)	1.17	0.25
CDC classification				
A	1 (6.7%)	8 (17.8%)		
B	2 (13.3%)	11 (24.4%)		
Severe B	4 (26.7%)	2 (4.4%)		
C	8 (53.3%)	24 (53.3%)		
HIV encephalopathy diagnosis	2 (13.3%)	7 (15.6%)		

Kaufman Assessment Battery for Children (KABC) Second edition composite scores

	HIV+children			
	After-12wk (> 12 weeks)	Before-12wk (12 weeks)	t or χ^2	p-value
Mental processing index	74.07 (5.08)	71.50 (8.63)	1.08	0.28
Non-verbal index	75.33 (9.93)	72.38 (11.15)	0.91	0.37

Values are N (% of total) or mean (standard deviation) except for age of first viral load suppression

[a] CD4 count at enrolment data were not available for 2 children.

[b] Age and duration of ART interruption mean and standard deviation based only on children in whom treatment was interrupted (N=28)

[c] where values are median (interquartile range (IQR)).

Table 3

Regression of regional brain volumes at age 7 years with HIV infection status.

Brain region	Volume (mm³)[a]		Regression controlling for sex			Regression controlling for sex and total intracranial volume		
	HIV+	Uninfected	Effect size (standard error)	p	FDR Adjusted p-value	Effect size (standard error)	p	FDR Adjusted p-value
Total intracranial volume	1427991 (104991)	1477778 (130776)	19199 (22288)	**0.02**[*]	0.09[†]	-	-	-
Global gray matter	713353 (52485)	731931 (63920)	24521 (14204)	0.07[†]	0.14	18518 (12489)	**0.01**[*]	**0.04**[*]
Global white matter	380733 (41701)	401041 (52381)	19685 (10213)	**0.01**[*]	0.09[†]	14388 (8229)	**<0.001**[*]	**0.01**[*]
Corpus callosum	3349 (450)	3462 (470)	76(89)	0.21	0.27	56 (86)	0.16	0.20
L caudate	3701 (501)	3807 (458)	98 (100)	0.26	0.30	50 (84)	0.19	0.22
R caudate	3868 (499)	3992 (451)	121 (104)	0.18	0.27	71 (87)	0.11	0.15
L hippocampus[1]	3443 (321)	3583 (362)	114 (89)	**0.03**[*]	0.11	97 (87)	**0.02**[*]	**0.05**[*]
R hippocampus[2]	3554 (358)	3696 (391)	184 (89)	**0.05**[*]	0.14	159 (85)	**0.03**[*]	0.06[†]
L putamen	5006 (526)	5205 (612)	371 (133)	0.06[†]	0.14	324 (123)	**0.03**[*]	0.06[†]
R putamen	5069 (538)	5313 (585)	360 (120)	**0.02**[*]	0.09[†]	326 (114)	**0.01**[*]	**0.04**[*]
L thalamus	7205 (647)	7365 (694)	-12 (125)	0.19	0.27	-81 (97)	0.09[†]	0.14
R thalamus	7151 (672)	7349 (731)	76 (134)	0.11	0.19	9 (110)	**0.05**[*]	0.08[†]
L lateral ventricle	6214 (2320)	6517 (2754)	-49 (498)	0.54	0.58	-291 (415)	0.51	0.54
R lateral ventricle	5731 (1937)	5870 (2458)	-127 (441)	0.75	0.75	-357 (354)	0.72	0.72

[a] Values are Mean (Standard deviation)

[1,2] Left and right hippocampus each had an extreme outlier, which was removed before analysis (N=101).

[*] p ≤ 0.05 (denoted in bold)

[†] p < 0.1